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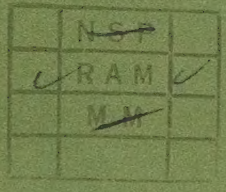

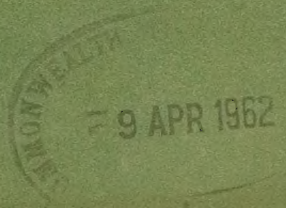

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НОМЕР ПОСВЯЩАЕТСЯ 10-ЛЕТИЮ ОСНОВАНИЯ БИОЛОГИЧЕСКОГО ИНСТИТУТА ЧЕХОСЛОВАЦКОЙ АКАДЕМИИ НАУК

В биологических науках, значение которых для будущей жизни человечества все более и более возрастает, было десять лет спустя очень важным возникновение Биологического института. В нем предоставлялась возможность сосредоточить и развить разнообразные биологические дисциплины, среди которых находится также экспериментальная ботаника.

Прошедшие пятнадцать лет после освобождения советскими войсками нашей республики являются периодом небывалого расцвета исследовательских работ в нашей стране. Это развитие, как качественное, так и количественное, можно с уверенностью считать результатом поддержки социалистического государства, которое всеми силами заботится о расширении основных, теоретических исследований во всех областях науки.

Можно с уверенностью считать, что основание нашего журнала «Биология плантарум», благодаря бескорыстной поддержке Биологического института Чехословацкой Академии Наук, принесло и ежедневно вносит свой вклад в дело мирного сотрудничества ученых всего земного шара.

АКАДЕМИК ИВАН МАЛЕК
Вице-президент ЧСАН, директор Биологического института

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THIS NUMBER MARKS THE 10TH ANNIVERSARY OF THE FOUNDATION OF THE BIOLOGICAL INSTITUTES OF THE CZECHOSLOVAK ACADEMY OF SCIENCES

The foundation of the Biological Institutes ten years ago was an important step in the development of the biological sciences in Czechoslovakia. The importance of these sciences for human life is constantly growing and the existence of the institutes under the auspices of the Czechoslovak Academy of Sciences has made it possible for research in many fields of biology, including experimental botany, to be concentrated and developed.

The fifteen years that have passed since the liberation of our Republic have been a period of unprecedented expansion in our scientific work. The fundamental advances, both qualitative and quantitative, have been acomplished thanks to the constant attention and support devoted by our socialist State to science, and above all to fundamental, theoretical research in all fields.

One of the results of the work accomplished by the Institute of Biology of the Czechoslovak Academy of Sciences has been the issuing of our journal BIOLOGIA PLANTARUM, which in the field of experimental botany makes its contribution to peaceful co-operation between scientists throughout the world.

ACADEMICIAN IVAN MÁLEK
Vice-president of the Czechoslovak Academy of Sciences, Director of the Institute of Biology

BIOLOGIA PLANTARUM (PRAHA)

2 (4) : 247—251, 1960

Changes in Sunflower Shoot Structure under Influence of Gibberellic Acid

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Department of Plant Physiology, Institute of Biology, Czechoslovak Academy of Sciences, Praha

Received January 20, 1960

Souhrn

Postřik 26denních rostlin slunečnice roztokem 50 γ kyseliny giberelové/ml vyvolal tvarové změny listů a statisticky průkazný vzrůst hodnoty délkošířkového indexu listů. Statisticky průkazně se zvýšil též počet listů na stonku za současného zvětšení hodnoty poměrů váhy sušiny stonků k váze sušiny listů.

Summary

The spraying of 26-day-old sunflower plants with a solution containing 50 μg. gibberellic acid/ml. brought about morphological changes in leaves and a statistically significant increase in the leaf shape index (length : width of leaves). The number of leaves on the stem also increased, together with a rise in the value of the ratio of dry weight of stems to dry weight of leaves.

Introduction

The influence of gibberellic acid (GA) is frequently manifested by an elongation of leaves and sometimes by a simplification of their shape. A considerable number of notes on this phenomenon can be found in the literature but only a few authors publish numerical data. The most striking effect of GA is that on the elongation of leaves and sometimes on the simplification of their shape. This effect was observed e.g. with rice (Hayashi, Takijima and Murakami 1953) with *Galinsoga parviflora* Cavan. (Knapp 1956), tomatoes (Gray 1957,

Rappaport 1957), cucumber (Kribben 1957), tobacco, bean, *Saintpaulia* and red pepper (Gray 1957), *Hedera canariensis* (Robbins 1957), *Phalaris canariensis* L. and *P. tuberosa* (Scurfield 1958), *Sesamum indicum* L. (Chakravarti 1958). Other authors call attention to the changes in leaf size irrespective of their shape (Marth, Audia and Mitchell 1956; Humphries 1958; Brian 1959). Some authors, working with other plants, observed an increase in the number of leaves, e.g. in the genus *Eucalyptus* (Scurfield and Moore 1958), in potato (Fischnich, Pätzold and Krug 1959).

In more extensive experiments the applicability of GA to sunflower under various conditions of mineral nutrition was investigated. On account of the great dispersion and lack of uniformity of literary data it is attempted in the present communication to call attention to some morphological deviations in the shoot structure which were treated statistically.

Material and Methods

Sunflower (*Helianthus annuus* L., var. Slovenská sivá) seeds were sown on June 8, 1959 into soil; on June 22, 1959, they were transferred in groups of three into flowerpots containing a mixture of three parts of river sand and one part of soil. On July 4, 1959, when the plants were 26 days old (about 5 leaves) mineral nutrients were added to the soil (var. A — no nutrients; var. B — 0·4 g. NH_4NO_3 per 1 kg. soil; var. C — 0·1 g. each of K_2HPO_4, KH_2PO_4, K_2SO_4 per 1 kg. soil, 0·4 g. NH_4NO_3 per 1 kg. soil). There were 22 flowerpots (each with three plants) for each of the above variants. Simultaneously with the addition of nutrients, one-half of the number of plants of each variant were sprayed with an aqueous solution of gibberellic acid of Czechoslovak production (Biotika, Slov. Lupča) at a concentration of 50 μg./ml. The solution adhered readily to the leaves even when no detergent was used. On August 13, 1959, i.e. 40 days after the addition of nutrients (and spraying with GA) the plants were harvested. The total length of stem, number of leaves, total dry weight of stems and leaves, maximum width and maximum length of the 3rd, 4th and 5th leaf (counting from the apex) were determined. The results were evaluated statistically by means of the *t*-test.

Results and Discussion

The application of GA considerably altered the morphological structure of sunflower plants in addition to its well-known effect on the longitudinal growth of stems. The experimental plants formed on an average three more leaves than the controls (Table 1). The leaves, however, had a deformed elongated shape (Fig. 1), were smaller in size and thinner. The change of leaf shape is best shown by the leaf-shape indices of the experimental and control plants (Table 2). Analogous changes in the leaf-shape index of *Ipomaea coerulea* were observed also by Njoku (1958).

The leaf deformations and the relatively lower inhibition of formation of stem tissues resulted in a change of the ratio of stem dry weight to leaf dry weight by some 50% (Table 2).

The statistical treatment of these morphological differences nearly always yielded highly significant values. A higher negative effect of GA was best

Fig. 1. Photographic prints of leaves of control (A) and GA-treated (B) plants.

marked in plants cultivated without nutrients when, apparently, the supply of plants with mineral nutrients did not correspond any more to the increased longitudinal growth. The total dry weight of plants treated with GA was lower, particularly in the unenriched variant (by 58%). There was no marked difference between the plants enriched with N (variant B) and those enriched with N, P, K (variant C).

An acceleration of development was observed in GA-treated plants after application of fertilizer — more plants flowered at the end of the experiment (var. B — 5 : 16, var. C — 7 : 19). GA was also observed to bring about ramification of higher parts of stems (5 or 6 plants of each variant).

Tab. 1. Length of stem and number of leaves of sunflower plants following a spray with 50 μg. GA/ml. For description of variants, see text

Variant	GA	Length of stem in cm.			Number of leaves		
		$\bar{x} \pm 3 . s_{\bar{x}}$	Difference caused by GA, in %	P	$\bar{x} \pm 3 . s_{\bar{x}}$	Difference caused by GA, in %	P
A	—	84·4 ± 3 . 2·95	+ 7,5	0·05	18·2 ± 3 . 0·64	+ 16·5	< 0·01
	+	90·8 ± 3 . 2·14		sign.	21·2 ± 3 . 0·82		highly sign.
B	—	83·4 ± 3 . 3,45	+ 18·2	< 0·001 highly sign.	18·8 ± 3 . 0·79	+ 19·1	< 0·01 highly sign.
	+	98·7 ± 3 . 2·63			22·4 ± 3 . 0·92		
C	—	89·3 ± 3 . 3·24	+ 3·5	0·55 in sign.	19·0 ± 3 . 0·72	+ 16·8	< 0·01 highly sign.
	+	92·4 ± 3 . 3·49			22·2 ± 3 . 0·82		

Tab. 2. Dry-weight index stem : leaves and average leaf-shape index of the 3rd, 4th and 5th sunflower leaves treated with 50 μg. GA/ml. For description of variants, see text

Variant	GA	Dry-weight index stem : leaves			Leaf-shape index of 3rd, 4th and 5th leaves		
		$\bar{x} \pm 3 . s_{\bar{x}}$	Difference caused by GA, in %	P	$\bar{x} \pm 3 . s_{\bar{x}}$	Difference caused by GA, in %	P
A	—	1·49 ± 3 . 0·051	+ 43·0	< 0·001 highly sign.	1·42 ± 3 . 0·023	+ 69·1	< 0.001 highly sign.
	+	2·13 ± 3 . 0·097			2·40 ± 3 . 0·035		
B	—	1·44 ± 3 . 0·059	+ 57·5	< 0·001 highly sign.	1·35 ± 3 . 0·016	+ 50·4	< 0·001 highly sign.
	+	2·27 ± 3 . 0·078			2·03 ± 3 . 0·049		
C	—	1·51 ± 3 . 0·053	+ 48·5	< 0·001 highly sign.	1·30 ± 3 . 0·018	+ 56·9	< 0·001 highly sign.
	+	2·24 ± 3 . 0·122			2·04 ± 3 . 0·047		

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Изменения в строении побега подсолнечника, вызванные гиббереллиновой кислотой

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Резюме

Орошением 26-тидневных растений подсолнечника раствором 50 μг/мл гиббереллиновой кислоты вызваны морфологические изменения листьев и статистически достоверный рост индекса длина / ширина у листьев. Статистически достоверно возросло также число листьев на стебле при одновременном повышении отношения сухого веса стеблей к сухому весу листьев.

BIOLOGIA PLANTARUM (PRAHA)

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Cytochemical and Electron-Microscopic Investigation of the Golgi Apparatus and Golgi-Resembling Inclusions in the Root Meristem of *Fagopyrum esculentum* Moench

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Souhrn

1. Klasickými cytologickými metodami, cytochemicky a elektronmikroskopicky byla studována cytoplasma pohanky. 2. Cytochemické studium inklusí podobných Golgiho aparátu bylo provedeno po fixaci Ca formolem a zalití do karbowaxu. 3. Byla vypracována charakteristika inklusí. Inkluse se podobají cytochemicky některým pigmentovým substancím živočišných buněk (lipopigmenty, fenolové pigmenty). 4. Byly studovány podmínky barvení inklusí basickými barvivy, sudanovou černí B a reakce kyselina jodistá—Schiffovo činidlo. 5. Funkční studium ukázalo podobný cyklus, jaký je znám při tvorbě sekretů v živočišných buňkách. 6. Golgiho aparát a jemu podobné inkluse se jeví pod elektronovým mikroskopem jako dvě různé složky.

Summary

1. The classical cytological methods, cytochemistry and electron microscopy were used to study the cytoplasm of *Fagopyrum esculentum* Moench. 2. Cytochemical investigation of inclusions resembling the Golgi apparatus was possible after fixation in Ca-formaldehyde and embedding in carbowax. 3. A characteristic of the inclusions was drawn up. The inclusions resemble some pigment substances of animal cells cytochemically (lipopigments, phenolic pigments). 4. Conditions of staining inclusions with basic dyes, Sudan black B and the PAS reaction were studied. 5. A functional investigation revealed a cycle resembling that known in the formation of secretions in animal cells. 6. The Golgi apparatus and inclusions resembling it appear under the electron microscope as two distinct components.

Introduction

In animal cells there exist numerous inclusions of a phenolic or lipid character (melanin, lipofuscin and others) which have been studied in recent years histochemically (Lillie 1957, Sacco, Castro and Pacheco 1958 and others). In plant cells pigment or pro-pigment substances (derivatives of anthocyans and flavones etc.) also occur which have received little histochemical attention so far (Reeve 1959). Milovidov (1959) describes phenolic inclusions in the root meristem of *Fagopyrum* and it was attempted here to investigate them in detail.

Material and Methods

Seeds of buckwheat (*Fagopyrum esculentum* Moench.) were germinated in Petri dishes at room temperature for 24 hours and roots removed and fixed. Three methods were used: 1. Classical cytological methods, 2. Cytochemical methods, 3. Electron-microscopical methods.

The cytological methods are described in the corresponding tables together with the results (Tables 1, 2).

In connection with the cytochemical reactions it was attempted to find the most suitable fixing fluid and embedding procedure. Bichromate methods are suitable enough but in some cases they block the active groups of the inclusions (Baker's calcium formaldehyde with chroming). Fixatives containing alcohol and acetic acid destroy the inclusions. Warm paraffin also partly destroys the phenolic and lipid inclusions of animal cells whereas polyethylene glycols (carbowax) are not injurious (Falck and Hillarp 1959). Baker's calcium-formaldehyde fixation (Ca-F) without chroming (24 hours) followed by embedding in carbowax was found to be most suitable. The inclusions remained well preserved and their histochemical reactivity was good. A freeze-drying method was also applied (Beneš and Hršel 1958). The histochemical methods are summarized in tables 3—12.

In connection with the electron-microscopic investigation three fixatives were tested: 1. OsO_4-uranyl acetate (Strugger 1956), 2. formaldehyde-OsO_4 (Sitte 1958), 3. $KMnO_4$ (Luft 1956). The objects were passed through alcohol and embedded in a mixture of butyl and methyl-methacrylate (Sitte 1958); sectioning was carried out on a Niklowitz ultramicrotome. The

Table 1. Impregnation methods for Golgi apparatus

Method	Inclusions	Mitochondria	Reference
Da Fano	+	—	Da Fano (WOLF 1954)
Aoyama	+	—	Aoyama (BAKER 1951)
Elftmann	+	—	Elftman (WOLF 1954)
Buffer, 1% OsO_4 pH = 7, 37° C 24 hours	+	—	PALADE 1952

Explanations: Tables of cytochemical reactions: — negative staining; (+) weakly positive staining; + positive staining; ++ markedly positive result.

sections were picked up on grids covered with formvar and carbon, inspected and photographed by means of an electron microscope Tesla and an electron microscope Siemens. The objects for comparison of light and electron microscopy were fixed in the same liquids and embedded in the same manner (methacrylate, paraffin).

Table 2. Methods for demonstration of mitochondria

Fixation	Staining	Inclusions	Mito-chondria	Others	Reference
Helly	Altman's acid fuchsin, tannin, methyl green	brown	red	starch bright green	Milovidov (GLICK 1949)
Helly	Benda's crystal violet	black	violet		Benda (KRAUSE 1926)
Zirkle	iron haemato-xylin	yellow-brown	black		O'Brien (HRŠEL 1956)

Table 3. Protein reactions

Fixation	Method	Inclusions	Mito-chondria	Others	Reference
10% formal-dehyde	Staining with buffered Amido-black 10 B pH 2·2 to 8·0	—	—	Cytoplasmic proteins are stained as expected at acid pH	BENEŠ 1960
10% formal-dehyde	Staining with fast green FCF pH 8	—	—	Nuclear chromatin bright green	ALFERT and GESCHWIND 1953
Ca-F	Staining with CrO_3 — eosin-light green	Brown-yellow	—	Cytoplasm green and pink, nucleolus violet	HRŠEL 1957
Formaldehyde, Navashin, Zirkle	Millon's reaction	Brown-red, partly destroyed	—	—	BAKER 1956 Bensley and Gersh (PEARSE 1953)
Freeze-drying Cold ethanol Ca-F	Freshly tetrazotized benzidine	Brown-orange partly destroyed	Violet-red	Cytoplasm violet	Danielli (LILLIE 1954)
Ca-F	Digestion by trypsin, staining with tol. blue	Blue, not destroyed	—	—	PEARSE 1953

Results

1. Cytological investigation by the classical methods.

In order to demonstrate the Golgi apparatus and the inclusions, they were impregnated with silver and osmium, yielding positive results throughout (Table 1). The mitochondria were not impregnated.

To demonstrate the mitochondria the objects were fixed and stained as shown in Table 2. The inclusions could be easily distinguished from the mitochondria both by their stainability and shape. For comparison (Milovidov 1957), fixation according to Navashin (acetic acid, chromic acid, formaldehyde) and staining with iron haematoxylin were used. As could be expected this method caused the destruction of the mitochondria and the inclusions were stained black.

2. Cytochemical analysis

a) Proteins. Proteins were detected by the methods shown in Table 3. Protein substances appear not to be present in considerable amounts in the inclusions. This is evidenced by the impossibility of staining the inclusions with acid dyes and by the resistance of the inclusions to trypsin digestion. Protein reactions based on the presence of tyrosine were positive.

b) Nucleic acids. Digestion with ribonuclease and extraction with hot trichloracetic acid do not bring about any decrease in the stainability of inclusions with toluidine blue, whereas the basophilia of the cytoplasm is completely removed. Nucleic acids probably do not occur in greater amounts in the inclusions.

c) Carbohydrates. The survey of results is shown in Table 4. After digestion with pectinase the cell walls are not stained with Alcian blue. The stainability with Alcian blue thus appears to be due to a pectin component. The reaction for ascorbic acid produces a number of small black granules mostly in the proximity of the cell walls.

The reaction of the inclusions and of polysaccharides with the HIO_4-Schiff reagent (McManus and Cason 1950) was carried out after previous blocking of various active groups, especially of aldehydes. The inclusions are positively stained with the Schiff reagent alone (without HIO_4). They are also intensively stained with Gomori's aldehyde-fuchsin after IIK or $KMnO_4$ oxidation (Culling 1957).

The results are summarized in Tables 5 and 6.

Digestion with β-glucosidase does not affect the reaction with the HIO_4-Schiff reagent in the inclusions.

d) Lipids. Lipids were detected by the methods summarized in Table 7. It follows from the table that the inclusions will be stained by lipid stains when fixed in calcium formaldehyde but not chromed. After fixation with Ca-F with chromation the mitochondria will be stained with Sudan black B and methazol blue. The stainability of the inclusions with Sudan black B was tested after blocking reactions (2% Fettschwarz "Geigy" in 70% alcohol 5 hours at 60° C; the objects were then rinsed in 70% alcohol, then in water and mounted in glycerol-gelatin). The results are summarized in Table 8.

In the blocking reactions the solvents without agents were used as controls. The fading of colour after blocking with 2,4-dinitrofluorobenzene and p-toluenesulphochloride is caused by the extraction of the inclusions with alkaline alcohol, as shown by the controls.

e) Phenols. Phenolic substances were detected in the inclusions by methods given in Table 9.

It follows from the table that the tests for phenols are positive. Attention was devoted to staining with toluidine blue. From a part of the sections nucleic acids were removed by extraction with hot trichloracetic acid, a part was left unextracted. The sections were stained for 30 min. in 0.1% buffered solutions of toluidine blue, passed through isopropyl alcohol and xylene in to Canada balsam. For results see Table 10.

For comparison see the results of staining inclusions with the acid dye Amidoblack 10 B in the same table.

The cause of basophilia of the inclusions was also investigated by the application of various blocking agents (Table 11).

Table 4. Reactions for polysaccharides

Component	Fixation and embedding	Method	Inclusions	Mitochondria	Others	Reference
1,2-glycols	Ca-F carbowax	HIO_4 Schiff	Violet brown-violet	—	—	MOWRY 1956
1,2-glycols	Formaldehyde, paraffin	HIO_4 Schiff	Violet, brown-violet partly destroyed	—	—	MOWRY 1956
Acid polysaccharides	Ca-F carbowax	Alcian blue	—	—	Cell walls and some granules in cytoplasm blue	MOWRY 1956
Acid polysaccharides, 1,2-glycols	Ca-F carbowax	Alcian blue-HIO_4 Schiff comb.	Violet, brown-violet	— —	Cell walls blue	MOWRY 1956
Acid polysaccharides, 1,2-glycols	Bouin paraffin	Alcian blue-HIO_4 Schiff comb.	Mostly destroyed	—	Cell walls blue	MOWRY 1956
Acid polysaccharides	Ca-F carbowax	Dial. iron	—	—	Cell walls and some granules in cytoplasm blue	Hale (PEARSE 1953)
Acid polysaccharides	Ca-F carbowax	Tannin-iron alum	—	—	Some light grey granules in cytoplasm	SALAZAR 1923
Ascorbic acid	Absolute alcohol	Silver nitrate acetic acid	—	—	Granules in cytoplasm, especially along cell walls	CHAYEN 1953
Pectin	Ca-F carbowax	Pectinase, Alcin blue-HIO_4, Schiff	Brown-violet violet,	—	Cell walls not stained with Alcian blue	PEARSE 1953

Table 5. Blocking of the PAS reaction in inclusions

Group	Blocking	Result	Note	Reference
Hydroxyl	Acetylation	—	Slight brown tinge of inclusions	BURSTONE 1955
	Pyridine control	+	Brown-violet colour of inclusions	BURSTONE 1955
Aldehyde	Phenyl hydrazine	—	Yellow colour of inclusions, polysacharide granules violet	PEARSE 1953
	Acetic acid control	(+)	Inclusions violet	PEARSE 1953
	Hydroxylamine	—	Brown colour of inclusions, polysaccharide granules violet	PEARSE 1953
	Sodium acetate control	(+)	Inclusions violet	PEARSE 1953
Carboxyl	Methanol, HCl	(+)	Brown and violet colour of inclusions, polysaccharide granules violet	LILLIE 1954
	Methanol control	+	Brown and violet colour of inclusions	LILLIE 1954

Table 6. Reactions of inclusions and polysaccharides

Reaction	Inclusions	Polysaccharide granules
Schiff's reagent alone (without HIO_4)	(+)	—
HIO_4-Schiff's reagent, without pre-blocking	+	—
HIO_4-Schiff's reagent, before reaction blocked by phenyl hydrazine, hydroxylamine and methanol with HCl	—	+

Table 7. Reactions for lipids

Fixation and embedding	Method	Inclusions	Mitochondria	Note	Reference on method
Ciaccio, paraffin	Sudan black B	+	—		LISON 1936
Ciaccio paraffin	Acid haematein	+	—		Baker (PEARSE 1953)
Ciaccio, paraffin	Methazol blue	+	—	Inclusions bright blue and red	PEARSE 1955
Ciaccio, paraffin	Fettrot	(+)	—	Inclusions light redbrown	PEARSE 1953
Ca-F, carbowax	Sudan black B	+	—		LISON 1936
Ca-F, carbowax, chroming	Sudan black B	—	+		LISON 1936
Ca-F, carbowax	Acid haematein	—	—		Baker (PEARSE 1953)
Ca-F, chroming, carbowax	Acid haematein	—	—		Baker (PEARSE 1953)
Ca-F, carbowax	Methazol blue	+	—		PEARSE 1955
Ca-F, chroming, carbowax	Methazol blue	—	+	Inclusions yellow-brown	PEARSE 1955
10% formaldehyde, paraffin	Sudan black B	+	—	Inclusions partly destroyed	LISON 1936
Ca-F, paraffin	Buffer OsO_4, 24 hr., 37° C	+	+		PALADE 1952 (modif.)
Ca-F, carbowax	Nile blue	+	—	Inclusions stained blue	Cain (PEARSE 1953)
Ca-F, chroming, carbowax	Nile blue	—	—	Inclusions yellow-brown	Cain (PEARSE 1953)
Controlled chromation	Sudan black B	+	—		Elftmann (PEARSE 1958)
Bouin, extraction with pyridine 24 hr. 60° C	Sudan black B	—	—	Relicts of inclusions	Baker (PEARSE 1953)
Bouin, extraction with pyridine 24 hr. 60° C	Methazol blue	—	—	Relicts of inclusions are stained intensely with methazol blue	Baker (PEARSE 1953 PEARSE 1955)
Ca-F, carbowax	Peracetic acid, Schiff's reagent	(+)	—		LANDING and HALL 1956
10% formaldehyde, paraffin	UV light, Schiff's reagent	—	—	Inclusions partly destroyed	LANDING and HALL 1956
Ca-F, carbowax	Mercury diphenyl carbazone	+	—		Okamoto (PEARSE 1958)

Table 8. Staining of inclusions with Sudan black B after blocking with various agents

Blocking	Result	Note	Reference on blocking
Acetylation	+		BURSTONE 1955
Phenyl hydrazine	+		PEARSE 1953
Hydroxylamine	+		PEARSE 1953
Methylation	+	Brown colour of inclusions	LILLIE 1954
Benzoylation	+	Intense staining of the whole section	PEARSE 1953
2,4-Dinitrofluorobenzene	(+)	Slight brown colour	BURSTONE 1955
Ethanol cont. NaOH	(+)	Slight brown colour	
p-Toluenesulphochloride	(+)	Slight brown colour	HERRIOT 1947
Ethanol cont. NaOH	(+)	Slight brown colour	
Ninhydrin	+		Monné and Slauterback (HRŠEL 1957)
Deamination	+		BURSTONE 1955
Peracetic acid	++		LANDING and HALL 1956
$KMnO_4$	—		LANDING and HALL 1956
CrO_3	—	Inner layers of cells positive, surface layers negative	From the reaction with ammoniacal silver for mucins (PEARSE 1953)
$HgCl_2$	+		From the reaction for plasmalogen (PEARSE 1953)
HIO_4	+		Longer modification (LANDING and HALL 1956)
HK	—		From the argentaffin reaction (PEARSE 1953)
Extraction of the stained preparation with diethyleneglycol	(+)		LILLIE 1954
Bromination	+		LILLIE 1954

Table 9. Reactions for phenols

Fixation	Method	Inclusions	Note	Reference on method
10% formaldehyde	Dianisidine	+	Inclusions brown-yellow and yellow	LILLIE 1954
10% formaldehyde	Nitroanisidine	+	Inclusions brown-yellow and yellow	LILLIE 1954
Freeze-drying, cold absolute alcohol, Ca-F	Diazotized saphranine	+	Inclusions orange-red, cytoplasm violetred	Lillie, Burtner and Henson (LILLIE 1954)
Zenker	Chromaffin reaction	+	Inclusions dark blue or greenblue, cytoplasm blue, nucleoli violet	Sevki (PEARSE 1953)
10% formaldehyde, Ca-F	Celestine blue, Toluidine blue	+ +	Inclusions dark-blue, cytoplasm blue and violet	CLARA 1934
Formaldehyde, ferrous sulphate	Ferrous sulphate	+	Inclusions black-green	JOHANSEN 1940
10% formaldehyde	Argentaffin reaction	+	Inclusions black	Masson-Fontana (PEARSE 1953)

Table 10. Staining at controlled pH

pH of the staining solution	Inclusions	Cytoplasm	Nucleolus	Nucleus
a. Staining with toluidine blue without extraction of nucleic acids				
2·2	+	—	—	—
3	+	+	—	—
5	++	++	+	—
6	++	++	++	++
7	+	++	++	++
8	+	++	++	++
b. Staining with toluidine blue after extraction of nucleic acids				
2.2	+	—	—	—
3	+	—	—	—
5	+	—	(+)	—
6	++	—	+	(+)
7	++	(+)	++	(+)
8	++	(+)	++	(+)
c. Staining with Amidoblack 10 B				
2.2	—	++	++	+
3	—	++	++	+
5	—	(+)	+	+
6	—	—	(+)	(+)
7	—	—	(+)	(+)
8	—	—	(+)	(+)

Table 11. Blocking of the staining of inclusions with toluidine blue

Blocking	Result	Note	Reference on blocking
Acetylation	—		BURSTONE 1955
Deacetylation with 0·1 N-KOH	++	Inclusions are stained very intensely	McMANUS and CASON 1950
Phenyl hydrazine	+		PEARSE 1953
Hydroxylamine	+		PEARSE 1953
Methylation	+	Cytoplasm is stained weakly	LILLIE 1954
Benzoylation	—	Nucleoli blue, cytoplasm is not stained	PEARSE 1953
2,4-Dinitrofluorobenzene	+		BURSTONE 1955
p-Toluenesulphochloride	+		HERRIOT 1947
Ninhydrin	+		Monné and Slauterback (HRŠEL 1957)
Deamination (HNO_2)	++	Inclusions black-blue	BURSTONE 1955
Peracetic acid	+		LANDING and HALL 1956
$KMnO_4$	++	Inclusions black-blue	LANDING and HALL 1956
CrO_3	(+)	In some cells inclusions are not stained, in others they are stained green	From the reaction with ammoniacal silver for mucins (PEARSE 1953)
$HgCl_2$	+		From the reaction for plasmalogen (PEARSE 1953)
HIO_4	++	Inclusions black-blue	Strong modification (LANDING and HALL 1956)
HK	—		From the argentaffin reaction (PEARSE 1953)

In all cases blocking reactions were carried out both on sections extracted by hot trichloroacetic acid and unextracted. Controls using solvents without agents were run simultaneously.

f) Tests for pigments. As the histochemical reactions show the inclusions could contain pigment-like substances. The corresponding tests are listed in Table 12.

The Lillie's test with Nile blue (BAYER) was negative. The inclusions were stained blue whereas methylated lipofuscins (i. e. esterified fatty acids) should be stained pink.

3. Electron-microscopic study

For the purpose of electron-microscopic investigation a neutralized solution of formaldehyde-OsO_4 (SITTE 1958) and potassium permanganate (LUFT 1956) were used as the most suitable fixatives. The first of them detects cytoplasmic membranes and the so-called Palade granules (Fig. 1), the second preserves the cytoplasmic membranes only (Fig. 2). After fixation with $KMnO_4$ it may be observed that an endoplasmic reticulum appears, composed of double mem-

Table 12. Rewiew of tests for pigments

Method	Application	Result	Note	Reference
Schmorl's ferric-ferricyanide test	Determination of lipofuscins and melanins	+	All inclusions are positive	PEARSE 1953
Hueck's test with Nile blue-H_2O_2	Distinction between melanins and lipofuscins	(+)	Inclusions in older cells are stained light blue	PEARSE 1953
Gomori's chromium haematoxylin	Lipofuscins	—		PEARSE 1953
Long Ziehl-Neelsen's test with carbolfuchsin	Acid-fast lipofuscins	+	Inclusions are stained bright red	PEARSE 1953
Lillie's Nile blue with methylation	Lipofuscins	—	Inclusions are stained blue instead of pink	LILLIE 1958
Pearse's alkaline tetrazolium (BT)	Lipofuscins and Sulphur or Sugars cont. substances	—		PEARSE 1958

branes (Fig. 5a); the same structures but with the Palade granules (Fig. 3d) can be observed in objects fixed with formaldehyde-OsO_4, as described by various authors (Buvat 1957, Sitte 1958, Heitz 1959). The mitochondria in objects fixed with $KMnO_4$ also possess the well-known structure (Fig. 2). In objects fixed with formaldehyde-OsO_4 the proplastids possess a more dense inner matrix than the mitochondria (Fig. 1). A non-osmiophilic component which could possibly correspond to starch can also be observed inside them. After fixation with osmium-containing fixatives the cells were found to contain only vacuoles with markedly osmiophilic relicts of inclusions. It appears that $KMnO_4$ preserves inclusions better than OsO_4. The filamentous shape of inclusions also better corresponds to the canalicular character found in some cells with the optical microscope (Fig. 7).

4. A functional view on the cells of the *Fagopyrum* root meristem

During the formation of inclusions and polysaccharide granules (see discussion) a similar cycle may be observed as described for the development of mitochondria, proplastids and spheroplasts in the embryos of *Triticum* and *Oryza* (Hršel 1957, Hršel 1959). During the first stages of development small granules can be observed in the active region adjacent to the nucleus (so-called Golgi zone, Plate 3k). Later, when the cell becomes differentiated, the small granules are found to merge to form filaments and later still a network. In this phase they resemble the Golgi apparatus of animal cells as revealed with the light microscope. During further development an expansion of the inclusions into the whole cell takes place. A similar process takes place during the formation of polysaccharide granules as was demonstrated on sections treated with phenylhydrazine, hydroxylamine, or methylated before the PAS reaction (cf. Discussion). During the earliest stages, fine polysaccharide granules may be observed here, too, immediately at the nucleus in the Golgi zone (Plate 3l). Later these granules grow in size and expand into the entire cell. In some cells the Golgi-resembling inclusions predominate, in others polysaccharide granules are formed to a greater extent and still in others both components are formed together.

Discussion

In a report at the Symposium of the German Histochemical Society (Kiel 1959) the first communication on the histochemical investigation of *Fagopyrum* inclusions was presented. The present paper represents a continuation of the study of this problem.

Classical methods (impregnation with silver and osmium) were used here to detect inclusions in the root meristem of *Fagopyrum*. Morphologically, the inclusions in meristems were unrecognizable (as revealed by light microscopy) from the Golgi apparatus of animal cells treated in the same manner. Therefore it was attempted to employ the cytochemical methods and electron microscopy for the elucidation of this question. It was found by cytochemical methods that the inclusions investigated here differ from the Golgi apparatus of animal cells in their content of phenolic substances and in some other characteristics as mentioned later. The Golgi apparatus of animal cells contains lipids, polysaccharides and proteins (Baker 1953, Lacy 1954).

The following reactions of the inclusions were selected as the basis of the cytochemical investigation: A. Periodic acid-Schiff reagent (polysaccharides), B. stainability with basic dyes (phenols and lipofuscins), C. stainability with Sudan black B (lipids).

A. The reaction of periodic acid-Schiff reagent (PAS) and other reactions for carbohydrates

The PAS reaction results in a violet-brown colour of the inclusions. Even with the Schiff reagent alone without oxidation a positive reaction of varying intensity is obtained. In order to distinguish the reaction of the Schiff reagent alone from the PAS reaction various blocking agents were applied (Lillie 1954, Pearse 1958) After the application of phenylhydrazine and hydroxylamine the inclusions do not give a positive PAS reaction. The inclusions are thus shown not to contain PAS-positive substances.

After treatment with phenylhydrazine, hydroxylamine, or methylation before the PAS reaction, granules of various size different from inclusions were detected. These granules probably contain polysaccharides (starch?) The PAS reaction alone (without previous blocking) will not cause these granules to stain. In addition, some other granules of different character were detected in several cells by staining for the detection of acid polysaccharides. Summarizing, the cells thus appear to contain three different components: 1. Inclusions, 2. after blocking reaction, the PAS-positive granules, 3. other granules stainable with Alcian blue and dialysed iron. The Schiff reagent alone will stain only the inclusions investigated.

B. Stainability with basic dyes

It is known from plant biochemistry (Klein 1933) that polyphenols (e.g. tannins) react with basic dyes (methylene blue and others). Clara (1934) employed stainability with mordant and basic dyes for the histochemical

detection of phenols in the tissue (the Clara reaction). This reaction is not specific as it stains not only phenols but also lipid pigments (Pearse 1953, p. 343) The Clara reaction, however, is used as a test for phenol and lipid pigment substances (melanins, lipofuscins) (Lillie 1957, Sasso, Castro and Pacheco 1958, Lillie, Weissbach and Glenner 1959, Falck, Hillarp and Torp 1959). The basophilic character of phenolic and lipid pigments contrasts with that of the cytoplasm in being quite marked even at lower pH values (Lillie 1957, Sasso, Castro and Pacheco 1958). According to our experiments, extraction with hot trichloracetic acid destroys the basophilia of the cytoplasm but the basophilic inclusions preserve their stainability

The Clara reaction is inhibited by acetylation, benzoylation and iodination. After deacetylation with 0·1 N-KOH a very intense colour of inclusions is produced. The stainability of inclusions also increases after deamination with HNO_2, oxidation with $KMnO_4$ or periodic acid.

C. Stainability with Sudan black B

In recent years some attention has been devoted to the staining of lipids with Sudan black B (Fredericsson 1958, Meier 1959 and others). It was attempted to investigate the cause of the stainability of the inclusions with Sudan black B. It was found that some oxidation agents, such as $KMnO_4$, iodine and partly CrO_3 inhibit the staining with Sudan black B, whereas the other reagents are devoid of any significant effect on the staining. Since it is known from the literature (Fredericsson 1958) that the lack of specificity of staining with Sudan black B can be due to its character basic the staining with Sudan black B and with toluidine blue were compared. The results show that the staining is not inhibited by the same reagents. After oxidation with $KMnO_4$ a very intense colour with toluidine blue is produced whereas the staining with Sudan black is inhibited. After benzoylation and acetylation the colour due to toluidine blue is inhibited while the stainability with Sudan black B is not affected. Iodine in the Lugol solution inhibits both staining processes.

Lillie (1954, p. 302) maintains that the extraction of Sudan black B-stained preparations with diethylene glycol should serve to distinguish between lipids from which the dye is extracted, and the so-called sudanophilic substances where the dye remains in the granule. The staining of inclusions with Sudan black B was partially reduced after this procedure.

These results cannot be generalized and the authors are aware of the exceptionality of the inclusions studied.

In *Fagopyrum* there exist three different types of staining with basic dyes: a) Staining of cytoplasm and nucleus with toluidine blue depending on the pH of the staining solution, b) staining of inclusions with toluidine blue independent of the pH of the staining solution resistant to hot trichloracetic acid extraction, c) staining of inclusions with alcoholic Sudan black B.

A comparison of the inclusions with the pigment substances of animal cells:

In animal objects there are two groups of pigments which can be compared with the inclusions studied here, namely phenolic pigments and lipopigments.

The first group contains pigments of the melanin and adrenochrome type; these substances are chromaffin, argentaffin and argyrophilic. They are stained with basic dyes and give the diazonium reaction and the Schmorl test. The PAS reaction is sometimes positive. Pearse (1953) is of the opinion that those pigments do not contain a carbohydrate component but are associated with unsaturated fats which react with the HIO_4-Schiff reagent. The other group of pigments are the lipopigments (lipofuscins, haemofuscins, cytolipochromes, pseudomelanic pigments, lipochromes, ceroids etc). They are stained with basic dyes. After oxidation with $KMnO_4$ the basophilia increases. They are PAS-positive and even the plasmal reaction is positive. They are stainable with Sudan black B and reduce ferricyanide. The Hueck test (Nile blue staining resistant to hydrogen peroxide) is positive.

Histochemical reactions of inclusions of *Fagopyrum* are similar to the reactions of the substances of both these groups

According to Gomori (1958) the lipopigments apparently contain polymeric and unsaturated fatty acids. Since the oxidation of these substances does not take place in the same way in all cells, different degrees of staining with basic dyes are obtained on the same section (the same may be observed on *Fagopyrum* inclusions).

Pearse (1953) maintains that lipopigments contain oxidation products of fats and fatty acids which brings about their reactivity with benzidine, leucomalachite green and the like. A positive PAS reaction does not necessarily imply that the substance contains a glycolipid Aldehydes can be formed from unsaturated lipids. At certain intermediary stages lipids can possess Schiff-positive groups. These substances are also acid fast (their staining due to basic dyes is resistant to differentiation by acids). The acid fast substances are unsaturated fatty acids of high molecular weight. They do not possess simple histochemical characteristics, i. e. all the mentioned reactions need not be positive for all types of these pigments. The same author describes two types of granules in the neurohypophysis, the first of these being stained with Sudan black B, PAS-positive, reducing ferricyanide, argentaffin and weakly basophilic, the second being markedly basophilic, acid fast, less reducing ferricyanide, less argentaffin, not stainable with Sudan black B and PAS-negative. He is of the opinion that we are dealing here with two varieties of the same pigment rather than with two pigments. Lillie (1954) describes a number of different types of pigments of a similar histochemical behaviour.

On the basis of these and the present authors' findings it appears that the inclusions studied in *Fagopyrum* are similar to these substances. Pearse (personal communication) mentions that the *Fagopyrum* inslusions are histochemically similar to the so-called pseudomelanin of the human intestine and appendix where the phenolic substances are dissolved in lipofuscin.

The following characteristic of *Fagopyrum* inclusions can be drawn up: 1. They are basophilic even at low pH values and after extraction of nucleic acids. 2. They are stained with Sudan black B and other lipid techniques. 3. The positive PAS reaction is probably not due to polysaccharides. 4. They react with the Schiff reagent alone with different intensity. 5. They reduce ferricyanide (the Schmorl test). 6. They are acid fast (the Long Ziehl-Neelsen test). 7. They are markedly argentaffin and argyrophilic. 8. They are stained yellow and brown-orange after the alkaline diazonium reaction. 9. They are stained blue with Nile blue. The Hueck test is weakly positive. 10. They are not stained with acid dyes. 11. They are mostly extractable with pyridine.

Morphologically, the animal cell pigments are granular but the *Fagopyrum* pigment forms canalicular structures resembling the Golgi apparatus in some cells and in older cells it fills the entire cell.

It is hoped that the methods used here will make it possible to study other pigments and tannin substances in plant tissues histochemically.

Electron microscopy. It was revealed by cytochemical investigation that inclusions resembling the Golgi apparatus cannot be related to Golgi structures

known from animal cells as their chemical composition is different. These findings were confirmed by electron microscopy.

Two structures were detected: 1. Lamellar structures of the Golgi apparatus (Dictyosomes Figs. 3a, 3b, 3e, 4), similar to these described by other authors in plant objects (Porter 1957, Buvat 1957, Perner 1957, Perner 1958, Heitz 1958, Sitte 1958, and others). 2. Vacuolar inclusions as stringlike intensely coloured structures, frequently divided into smaller particles after fixation with $KMnO_4$ (Fig. 7).

It would perhaps be possible to postulate a formation of these structures with the participation of ergastoplasmic membranes (Fig. 5a). Occasionally, two parallel filamentous structures can be observed in the cytoplasm which are different from the normal ergastoplasmic structures (Fig. 5b). Sometimes the ergastoplasmic membranes are arranged in circles (Fig. 6). These findings may correspond to the first phases of development of inclusions. Other authors share the opinion that the formation of secretions is associated with the ergastoplasm (Woihlfarth-Bottermann and Moericke 1959). Buvat 1958 describes the development of plant vacuoles from the membrane and tubular system of the ergastoplasm. He also mentions that the elongated vacuoles formed by dilatation of ergastoplasmic sinuses correspond to the vacuolar apparatus which becomes hypertrophic due to the action of neutral red as described earlier. But in animal objects, Lacy and Challice (1956) and Dalton and Felix (1956) use impregnation with osmium and silver for electron microscopy to establish the identity of the lamellar Golgi structures with the impregnable structures observed with the light microscope. The impregnable canalicular structures in plant cells (Bensley 1910) described earlier as the Golgi apparatus or vacuoles must be studied further.

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Цитохимическое и электронномикроскопическое исследование аппарата Гольджи и на аппарат Гольджи похожих включений в меристемах корешков гречихи *Fagopyrum esculentum* Moench

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Резюме

Цитоплазма гречихи изучалась нами классическим цитологическим, цитохимическим и электронномикроскопическим методами. Цитохимическое изучение включений, похожих на аппарат Гольджи, обусловлено применением метода фиксации Са-формальдегидом и заливкой в карбоваке. Выработана характеристика включений. Включения цитохимически похожи на пигментные субстанции животных клеток. Изучен механизм окраски включений основными красителями и суданом черным и PAS-реакцией. Изучение с функциональной точки зрения выявило цикл, известный для образования секретов в животных клетках. Аппарат Гольджи и похожие на аппарат Гольджи включения кажутся под электронным микроскопом двумя независимыми образованиями.

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Veränderungen des Wachstums und der Atmungsvorgänge von Maispflanzen, hervorgerufen durch erhöhte Dichte der Bestände

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Souhrn

K pokusům jsme použili kukuřici Hodonínský koňský zub žlutý. Předem naklíčené obilky byly vysety v pěti různých hustotách do Mitscherlichových nádob s pískem. Každá série byla pětkrát opakována. Kukuřičné rostliny byly kultivovány 21 dnů. Byly sledovány změny v růstu, v obsahu vody, v aktivitě katalásy a peroxydásy a v intensitě dýchání.

Pokusy ukázaly, že vlivem stoupající hustoty porostu se spolu s růstem podstatně oslabovaly i fysiologické pochody uvnitř rostlin. Tyto procesy byly intensivněji oslabovány v kořenech než v nadzemních částech. Nejvíce ze všech sledovaných fysiologických pochodů se snižovalo dýchání kořenů. S tím pravděpodobně souvisí snížení ostatních sledovaných fysiologických funkcí, mimo jiné i zmenšený příjem minerálních látek z půdy, jak bylo ukázáno v předchozí práci autora (1959).

Zusammenfassung

Für die Versuche haben wir die Maissorte Hodonínský koňský zub žlutý verwendet. Die vorgekeimten Getreidekörner wurden in fünf verschiedenen Dichten in die mit Sand gefüllten Mitscherlich-Gefässe ausgesäht. Jede Serie wurde fünfmal wiederholt. Die Maispflanzen wurden 21 Tage lang kultiviert. Es wurden Veränderungen des Wachstums, des Wassergehalts, der Katalase- und Peroxydase-Aktivität sowie der Atmungsintensität festgestellt.

Die Versuche erwiesen, dass sich unter dem Einflusse einer steigenden Bestandesdichte im Zusammenhang mit dem Gesamtwachstum auch die physiologischen Vorgänge innerhalb der Pflanzen wesentlich schwächten. Diese

Prozesse wurden in den Wurzeln stärker als in den oberirdischen Teilen geschwächt. Unter allen beobachteten Vorgängen wurde die Wurzelatmung am stärksten herabgesetzt. Damit hängt wahrscheinlich auch die Herabsetzung der übrigen beobachteten physiologischen Funktionen zusammen, unter anderem auch die Herabsetzung der Mineralstoffaufnahme aus dem Boden, wie in der vorhergehenden Arbeit des Autors gezeigt worden ist (1959).

Einleitung und Problematik

Kulturpflanzen züchtet man stets in Beständen von bestimmter Dichte, die für den wirtschaftlichen Ertrag optimal ist und grösstenteils vom biologischen Charakter der gegebenen Pflanzenart abhängt. Eine zu hohe oder zu niedrige Bestandesdichte äussert sich gewöhnlich in einer Herabsetzung des wirtschaftlichen Effektes. Bei niedrigeren Dichten ist dies begreiflicherweise durch die kleine Anzahl von Individuen auf der Flächeneinheit bewirkt, während sich bei zu hohen Dichten ungünstige Lebensbedingungen für die Pflanzen bilden, die auf ihren Wuchs hemmend wirken.

Nach den Angaben mehrerer Autoren äussert sich die Wirksamkeit der verdichteten Bestände nicht nur in Wachstumsveränderungen, sondern auch in morphologischen und sonstigen biologischen Veränderungen der Pflanzen (Ivanovskaja 1948, 1950, Zavadskij 1954).

In unserer vorhergehenden Arbeit, in der wir diese Fragen untersuchten (F. Plhák 1959, siehe dort auch ausführlichere Literatur), haben wir festgestellt, dass es unter der Einwirkung einer erhöhten Bestandesdichte ausser den angegebenen Veränderungen auch zu Veränderungen innerer Prozesse des Pflanzenkörpers kommt. In Weizen- und Kornpflanzen veränderte sich im Verlaufe einer Vegetation deutlich der Gehalt an Phosphor, Chlorophyll und auch Wasser. Diese Veränderungen stimmten insgesamt mit den Wachstumsveränderungen überein und zeigten einige charakteristische Merkmale der Reaktion der Pflanzen auf die Erhöhung der Dichte. Sie wurden allerdings nur an den oberirdischen Teilen der Pflanzen verfolgt und liessen darum keine Beurteilung der Gesamtreaktion der Pflanzen auf die angegebenen Bedingungsveränderungen zu. Deshalb haben wir in dieser Arbeit die Einwirkung der erhöhten Bestandesdichte auf die physiologischen Prozesse und zwar sowohl der oberirdischen Teile als auch der Wurzeln beobachtet. Ausser Wachstum und Wassergehalt verfolgten wir auch die Atmungsprozesse, die nach Lvov (1952) ein besonders adäquater Indikator der Lebensintensität der Pflanzen sind. Zum Zwecke einer tieferen Erkenntnis der Atmungsprozesse haben wir auch die Aktivität einiger Oxydationsenzyme festgestellt.

Material und Methode

Für die Versuche wurde die Maissorte Hodonínský koňský zub žlutý aus der Ernte 1957 verwendet. Die vorgekeimten Körner wurden in Mitscherlich-Gefässe in verschiedenen Dichten ausgesäht und zwar zu je 10, 30, 50, 70, und 90 Getreidekörnern in einem Gefasse.

Jedes Gefäss hatte 20 cm Durchmesser, 21 cm Höhe und hatte einen Gesamtinhalt von 6,6 Litern. Als Zuchtsubstrat wurde Flussand benutzt. Jede Serie wurde fünfmal wiederholt.

Der Versuch verlief vom 10. 6. bis zum 2. 7. 1958. Mitscherlichs Gefässe mit den Versuchspflanzen wurden frei im Garten aufgestellt. Ausser der natürlichen Bewässerung durch Regenfälle wurden die Pflanzen einmal in der Woche mit 750 ml Sachsscher Nährlösung begossen.

Die Maispflanzen wurden in der Phase der vier Blätter bearbeitet. Es wurde das durchschnittliche Frischgewicht der ganzen Pflanzen, sowie das Frischgewicht und die Trockensubstanz der oberirdischen Teile und der Wurzeln gesondert für sich bestimmt. Aus jeder Serie wurden 25 Pflanzen einzeln gewogen. Weiter wurde die Atmungsintensität von Blättern und Wurzeln der Dichten von 10, 50 und 90 Pflanzen bestimmt. Die Wurzeln der Pflanzen wurden durch Auswaschen des Sandes in strömendem Leitungswasser zubereitet. Die Atmung wurde manometrisch in Warburgs Apparat mit Sondergefässchen von 100 ml Volumen bestimmt. In diese Gefässchen wurden je 3 g Frischmaterial eingewogen. Die Blätterproben wurden frei in die Gefässchen eingelegt, die Wurzelproben wurden in je 10 ml Sachsscher Nährlösung eingetaucht. Das Volumen der Blattproben wurde pyknometrisch bestimmt. In die Gefässchen wurde weiter gesondert je 1 ml 15% KOH-Lösung pipettiert. Die Atmung wurde bei 25° C gemessen und zwar ohne dass Licht hinzutreten konnte. Aus jeder angeführten Versuchsserie wurde die Atmungsintensität dreier Proben ermittelt. In den Pflanzen der angegebenen drei Serien wurde weiter die Aktivität zweier Oxydationsenzyme bestimmt. Die Aktivität der Katalase wurde manometrisch (Kleinzeller, Málek, Vrba, 1954) unter Verwendung von Phosphatpuffer mit einem pH 6, 9 bei 25° C bestimmt. Die Anfangskonzentration von Wasserstoff-Peroxyd betrug in 3 ml Substrat 0,1 Mol., die Konzentration der Pufferlösung 0,15 Mol. Die Aktivität der Peroxydase wurde kolorimetrisch nach der Methode Bach-Zupková (1921) eingerichtet von Tichý (1956) bei Laboratoriumswärme bestimmt.

Versuchsergebnisse

Das Frischgewicht der Maispflanzen sank unter dem Einfluss der erhöhten Anzahl von Pflanzen in den Gefässen allmählich ab. Bis zu einer fünffachen Verdichtung sank das Frischgewicht im Verhältnis zur Dichte bedeutend intensiver. Das Frischgewicht der oberirdischen Teile setzte sich weniger stark herab als das Frischgewicht der Wurzeln; bei der stärksten verwendeten Dichte machte dieser Unterschied 17% aus, wie aus Abb. 1 hervorgeht.

„Ähnlich wie das Frischgewicht, wurde auch die Trockensubstanz der Pflanzen unter dem Einfluss der Dichte herabgesetzt (siehe Abb. 2). Wiederum sank das Trockengewicht der Wurzeln stärker als das Trockengewicht der oberirdischen Teile, auch wenn die Unterschiede nicht so ins Auge fallen wie beim Frischgewicht.

Der Wassergehalt erlitt ebenso Veränderungen und zwar mit zunehmender Dichte ausserordentlich regelmässig (siehe Abb. 3), auch wenn es sich hier nicht um grosse Veränderungen handelte. In den oberirdischen Teilen der Maispflanzen zeigte der Wassergehalt unter dem Einfluss der höheren Dichte aufsteigende, in den Wurzeln aber im Gegensatz dazu absteigende Tendenz.

Die Atmung der Blätter setzte sich bei Verstärkung der Dichte fast linear herab, bei höchster Dichte um 23% (siehe Abb. 4). Zusammen mit der Atmung wurde auch die Aktivität von Katalase und Peroxydase in den Blättern herabgesetzt. Die Peroxydase sank nur unbedeutend, die der Katalase bis zu 21% (siehe Abb. 5).

Die Intensität der Wurzelatmung wurde durch die Dichte am meisten geschwächt. Sie sank fast linear mit steigender Dichte der Pflanzen und erwies ein Maximum des Absinkens von 74% im Vergleich zur lockersten

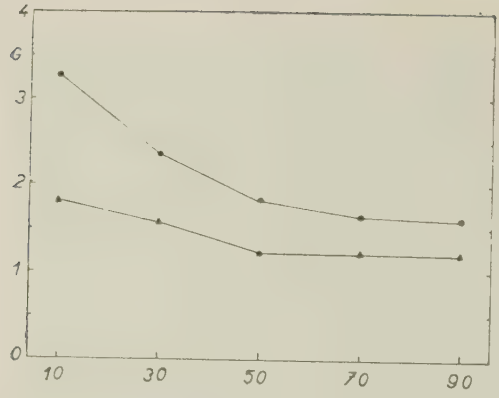

Fig. 1.

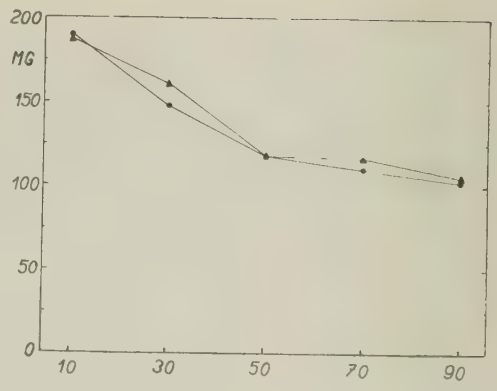

Fig. 2.

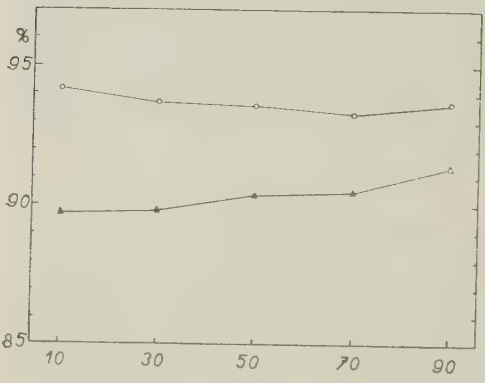

Fig. 3.

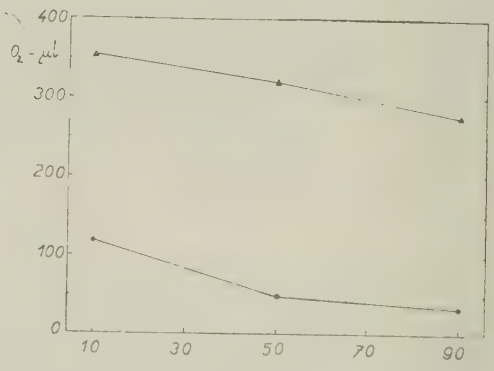

Fig. 4.

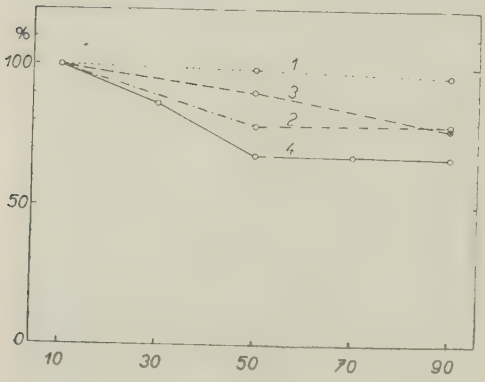

Fig. 5.

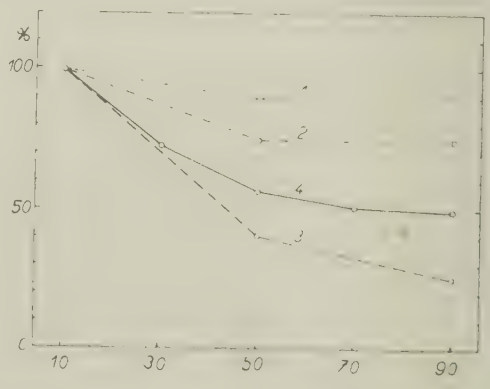

Fig. 6.

Abb. 1—4. Einfluss der Kultivierungsdichte auf die Veränderungen des Frischgewichtes (Abb. 1), der Trockensubstanz (Abb. 2), des Wassergehaltes (Abb. 3) und der Atmungsintensität (Abb. 4) der oberirdischen Teile — ▲ — ▲ — und der Wurzeln - ● - ● - ● der Maispflanzen. Kultivierung im Verlaufe von 21 Tagen

Dichte (siehe Abb. 4 und 6). Ebenso sank die Peroxydase-Aktivität in den Wurzeln intensiver als in den Blättern. Die Herabsetzung der Katalase-Aktivität der Wurzeln war ähnlich intensiv wie die der Blätter.

Bei einer statistischen Wertung waren die Unterschiede in Wachstum und Atmung beweiskräftig und zwar überzeugend beweiskräftig, die Unterschiede in der Katalase-Aktivität lagen nahe der Grenze der Deutlichkeit.

Diskussion

Aus den Ergebnissen der vorliegenden Arbeit geht klar hervor, dass die physiologischen Veränderungen sowohl der oberirdischen Teile, als auch der Wurzeln ähnlichen Charakter haben. Nahezu alle Werte der beobachteten Kennzeichen sanken allmählich unter dem Einfluss der steigenden Dichte. Das erweist, dass die gesamte Herabsetzung des Wachstums unter den vorliegenden Bedingungen von einer Herabsetzung der Aktivität der meisten physiologischen Vorgänge begleitet wird.

Die Intensität der Schwächung der einschlägigen Vorgänge in den Wurzeln und in den oberirdischen Teilen war aber nicht gleich. Aus den Veränderungen des Wachstums, der Atmungsvorgänge und der Aktivität der Enzyme in Abb. 5 und 6. ersieht man klar, dass die meistens beobachteten physiologischen Vorgänge in den Wurzeln stärker herabgesetzt wurden als in den oberirdischen Teilen der Maispflanzen. Eine mögliche Erklärung kann man wahrscheinlich darin suchen, dass sich durch eine Erhöhung der Dichte in der Anfangsphase der Wachstumsvorgänge die Bedingungen der Bodenverhältnisse stärker veränderten, die vor allem aufs Wurzelsystem einwirkten. Ebenso konnte hier die empfindlichere Reaktivität der Wurzeln auf die verschlimmerten Bedingungen im Vergleiche zu den oberirdischen Teilen eine wichtige Aufgabe erfüllen. Eine ähnliche Tatsache stellt zum Beispiel Ivanovskaja (1950) fest.

Aus den beobachteten physiologischen Vorgängen ist weiter ersichtlich, dass insbesondere die Wurzelatmung intensiv betroffen worden ist. Sie erniedrigte sich um 25% tiefer als das Wurzelwachstum. Die in dieser und in der vorhergehenden Arbeit beobachteten übrigen Faktoren wie Wassergehalt, Phosphor- und Chlorophyllgehalt setzten sich weniger herab (F. Pláh. 1959). Ebenso wurde die Aktivität von Katalase und Peroxydase, die

in Mitscherlichs Gefässen mit Sand. Achse x: Anzahl der Pflanzen im Gefäss. Achse *y*: Werte der beobachteten Kennzeichen. Die Atmungsintensität wird durch den Verbrauch von μl O_2/g Frischgewicht in 60 Minuten ausgedrückt.

Abb. 5—6. Vergleich der Einwirkung der steigenden Kultivierungsdichte auf die physiologischen Vorgänge in den oberirdischen Teilen (Abb. 5) und in den Wurzeln der Maispflanzen (Abb. 6).

Kurve 1 — Aktivität der Peroxydase, Kurve 2 — Aktivität der Katalase, Kurve 3 — Atmungsintensität, Kurve 4 — Frischgewicht der Organe.
Achse *x*: Anzahl der Pflanzen im Gefäss.
Achse *y*: Werte der verfolgten Kennzeichen ausgedrückt in Prozenten der Kontrolle.

Teile des enzymatischen Apparates der Atmungsprozesse der Pflanzen sind, schwächer herabgesetzt. Dieses Missverhältnis ist vielleicht durch die beträchtliche Kompliziertheit des Enzymapparates der Atmungsprozess erklärbar (siehe Lvov (1952), James (1954)) und dadurch, dass die einzelnen Enzyme hier nur Teilfunktionen zu erfüllen haben.

Dabei erfüllen die Atmungsvorgänge eine hochwichtige energetische Funktion im ganzen pflanzlichen Metabolismus. Insbesondere ist das Atmen in den Wurzeln eine Energiequelle, die für die Aufnahme von Mineralstoffen aus dem Boden notwendig ist. Eine Herabsetzung der Atmungsintensität, besonders auffällig bei den Wurzeln, musste darum eine bemerkenswerte Herabsetzung der Mineralstoffaufnahme aus dem Boden zur Folge haben, was schon in der vorhergehenden Arbeit (F. Plhák, 1959) am Beispiel des Phosphors gezeigt worden ist. Von der Intensität dieser Vorgänge sind auch die synthetischen Vorgänge unmittelbar abhängig. Oparin und Gel'man (1952) führen zum Beispiel an, dass sich bei der Schwächung der Atmung immer auch der Prozess der Synthese herabsetzt. Aus den angeführten Gründen ergibt sich also die Möglichkeit, in starkem Masse die Schwächung der Maispflanzen mit der festgestellten Herabsetzung der Intensität ihrer Atmungsprozesse in Zusammenhang zu bringen.

Welche unmittelbaren äusseren Ursachen die Herabsetzung der physiologischen Vorgänge und speziell der Wurzelatmung bewirkten, kann aus den angegebenen Ergebnissen schwer gefolgert werden. Man muss hier vor allem an die ungenügende Menge von Nährstoffen im Boden denken welche die meisten Autoren für eine der Hauptursachen der Schwächung der Pflanzen unter den angeführten Bedingungen halten (Ivanovskaja 1950, Zavadskij 1954). Mit dieser Behauptung lassen sich allerdings manche unserer Ergebnisse nicht ganz in Einklang bringen, vor allem jene nicht, welche die festgestellte Herabsetzung der Atmungsintensität betreffen. Nach den Angaben einiger anderer Autoren ruft allein die Herabsetzung des Gehaltes an Mineralstoffen im Boden im Gegenteil eine erhöhte Atmungsintensität der Wurzeln wie der oberirdischen Teile der Pflanze hervor. Solche Ergebnisse sammelten zum Beispiel Gregory und Sen [1937, zitiert nach Lvov (1952)] bei Gerste und nach Jastrebov (1956) bei Weizen. Andere Autoren führen allerdings auch gegenteilige Ergebnisse an, so zum Beispiel Dorochov (1956).

Von den anderen Bodenfaktoren könnte hier auch der Mangel an den übrigen Stoffen eine wichtige Rolle spielen, beispielsweise ein Mangel an zugänglichem Sauerstoff im Boden, ferner die spezifische Wirksamkeit der Wurzelmikroflora und ebenso die Einwirkung einer höheren Konzentration metabolistischer Ausscheidungen der Wurzeln der Versuchspflanzen.

Von den oberirdischen Faktoren muss man vor allem die Herabsetzung der Lichtintensität beachten, auch wenn sich bei unseren Versuchen bei der kurzen Wachstumsvergrösserung ein Lichtmangel nicht geltend machen konnte.

Alle diese Mutmassungen muss man in weiteren Versuchen prüfen.

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Изменение роста и дыхательных процессов в растениях кукурузы, вызванные повышенной густотой посева

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Резюме

Опыты проводились на кукурузе сорта Годонинский конский зуб желтый. Проросшие зерна высевались в пяти разных густотах в сосуды Митчерлиха с песком. В каждой серии было пять повторностей. Растения кукурузы выращивались 21 день. Нами были исследованы изменения роста, содержания воды, активности каталазы и пероксидазы и интенсивности дыхания.

Опыты показали, что по мере нарастания густоты посева вместе с ростом существенно ослаблялись также физиологические процессы в растениях. Это проявлялось более интенсивно в корнях, чем в надземных частях. Из исследованных физиологических процессов наиболее снижалось дыхание корней. С этим, вероятно, связано снижение других исследованных физиологических функций, кроме прочего, также пониженный прием минеральных веществ из почвы, как это было показано в предшествующей работе автора (1959).

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Wheat Striate Virus

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Souhrn

Na ochuravění obilnin, jež se kalamitně vystupňovalo na Českomoravské vysočině v letech 1954 až 1956, se podílel vedle hlavního původce těžkých škod viru sterilnosti a zakrslosti ovsa (VSZO) ještě virus proužkovitosti pšenice (VPP). Studium symptomatologie ukázalo, že VPP se u hostitelských obilnin projevuje charakteristicky odlišně od VSZO. VPP je však podobně jako VSZO specificky rozšiřován křísem *Calliyypona pellucida* Fabr. Při dlouhodobé individuální testaci vektorů pocházejících z lokality zamořené VSZO a VPP byly rozlišeny podle patogenních účinků na obilí čtyři skupiny hmyzu: 1. nepatogenní, 2. vyvolávající příznaky VSZO, 3. vyvolávající příznaky VPP a 4. vyvolávající většinou příznaky VSZO, ale občas též VPP anebo obou těchto virů současně. VPP je ve svém přenašeči persistentní a je přenášen jak larvami různých vývojových stupňů, tak i imagy *C. pellucida* obojího pohlaví. VPP býval přenášen méně pravidelně než VSZO. Mezi jednotlivými křísy byly značné diference v přenášečské účinnosti pro VPP, takže lze předpokládat existenci v tomto směru různě efektivních ras. V polních populacích u nás převládají patrně méně účinné rasy. K přenesení VPP bylo třeba minimálně devítihodinového pobytu vektora na hostiteli, zdá se však, že by stačila i kratší doba. Extrakty z rozdrcených listů proužkovitých pšenic, jakož i z infekčních křísů inokulovány do rostlin mechanicky (vtíráním, injekcemi) nezpůsobily nákazu. Pokusy o přenos půdou a kokotící (*Cuscuta campestris* Yunck.) byly negativní. Termický bod inaktivace viru v živém přenašeči se nepodařilo stanovit. V SSSR popsané viry „zakuklivanije" obilnin a ruská mosaika ozimé pšenice se liší od VSZO a VPP mimo jiné též produkováním typických inklusí a tím, že je lze snadno přivést ke krystalisaci.

Summary

In addition to the oat sterile-dwarf virus (OSDV) as the main agent causing grave damage to cereal crops in the Bohemian-Moravian Highlands during 1954—56, when the disease reached catastrophic proportions, it was found

that wheat striate virus (WSV) also played a part. Symptomatological study showed that there are characteristic differences between the effect of WSV on the host plants and that of OSDV. Similarly to OSDV, however, WSV is specifically spread by the leafhopper *Calligypona pellucida* Fabr. Following long-term individual testing of vectors taken from localities infested with OSDV and WSV, four groups of insects were distinguished according to their pathogenic effects on cereal crops: 1. nonpathogenic, 2. producing OSDV symptoms, 3. producing WSV symptoms, 4. producing for the most part OSDV symptoms, but occasionally WSV or symptoms characteristic for both viruses simultaneously. WSV is persistent in its vector and is transferred by *C. pellucida* larvae at different stages of development and by imagos of both sexes. WSV tends to be transferred less regularly than OSDV. Considerable differences in efficiency of carrying WSV are to be found between individual leafhoppers, so that it is possible to assume the existence of races with varying efficiency in this respect. Among field populations in this country less efficient races prevail. For the transmission of WSV to be effected it was necessary for the vector to remain on the host for at least nine hours, but it seems that a shorter period might suffice. Extracts of crushed leaves of wheat plants suffering from the striate disease and from infective leafhoppers failed to cause infection when inoculated mechanically (by rubbing, injection) into plants. Attempts to achieve transmission through the soil or by dodder (*Cuscuta campestris* Yunck.) gave negative results. It was not possible to determine the thermal inactivation point of the virus in the live vector. The mosaic (zakuklivanie) virus of oat and the Russian mosaic of winter wheat as described in the U.S.S.R. differ from OSDV and WSV among other points by the production of typical inclusions and by the fact that they can easily be induced to crystallize.

Introduction

It has already been reported in short notes (Růša 1958, 1959a, 1959b, 1959c, Růša et al. 1959) that during investigation of the catastrophic oats disease, which reached particularly serious dimensions in the south-western parts of the Bohemian-Moravian Highlands in 1954—56, in addition to oat sterile-dwarf virus (OSDV) as the main factor causing grave damage to crops, wheat striate virus (WSV) was also identified. It was reported that both viruses have the same vector, the leafhopper *Calligypona pellucida* Fabr., in nature. They are persistent in this vector and WSV can be transmitted transovarially in a high percentage of cases by infected females to their offspring, which was not observed in the case of OSDV.

This paper deals with the part of the experiments which led to the discovery of WSV in Czechoslovakia, to the elucidation of its symptomatology in the main host plants and to the investigation of some of its characters.

Signs of WSV were observed as early as 1956. They were dealt with in more detail in 1957, when very striking cases of striated plants were found in winter wheat in a field next to that on which oats had been completely infested

with OSDV the year before. This was in the experimental locality Sázava, the focal area of the OSDV epidemic in the Pelhřimov district. The character of the distribution of striated wheat in the crop (high degree of infection confined to the part of the field in the close proximity of the previous year's oat crop and a sharp drop in the proportion of diseased plants in a direction away from the previous oat field and deeper into the wheat field) indicated that the factor causing these symptoms in wheat was identical with that which had caused grave damage to oats (PRŮŠA 1957). Experiments showed, however, that the striation was produced by another virus, which was then named from the most typical sign on the first host plant on which it was identified, wheat striate virus. The remarkable type of distribution of the striated plants in the wheat crop was explained as being due to WSV having the same vector as OSDV; this vector gave preference to oats, but passed over to winter wheat when the remaining oats, which is cultivated here as a spring crop, died out completely during the autumn and winter.

Material and Methods

In the first experiment with WSV striated winter wheat found at the beginning of June 1957 near the village of Sázava was used as the source of infection. Oat seedlings variety Valečovský bílý were used as test plants. Infectivity was tested by three groups of insects: a mixture of aphid species caught on corn at Sázava, a mixture of bug species (Heteroptera) from the same source and a mixture of leafhopper species from a mixed crop of barley and oats at Ruzyne near Prague. Each group of insects was first left for three days' acquisition feeding on striated wheat and then fed for a week on healthy oats plants. Cages formed of two curved hard brass wires placed at right angles to each other and covered with white Silon stockings (BRČÁK 1959) were used in order to isolate the insects on the plants. The insects were killed at the conclusion of the test-feeding by spraying with the nicotine preparation Floron. Further particulars about this experiment are given in Table 1.

The question of the transmittability of WSV by different aphid species, caught on corn and grass fodder plants in the area of the maximum occurrence of WSV and OSDV, to the different corn crops was simultaneously examined in a series of similar tests. Further reference is not made to the results obtained, as they were negative.

After it had been established that the spread of virus signs is specifically related to the leafhopper *Calligypona pellucida* Fabr., the method of mass application of insects to groups of plants was replaced by the process of long-term individual testing of each leafhopper separately for a series of receptive plants (for details see PRŮŠA et al. 1959).

The insects used in the main experiment were caught at the edges of fields upon which there had been a completely infested crop of oats the year before (1957). The locality was Sázava. The insects were transferred by means of an entomological exhaustor, which had been modified for the purpose (Fig. 1) so as to allow both the necessary freedom of manipulation when catching the leafhoppers in special isolation cages of strong brass wire and Silon fabric (see illustration in PRŮŠA et al. 1959), and also rapid and careful transference of the insect from plant to plant. The main alteration was that instead of attaching the insect-isolating fabric to the tube, which with its rubber extension is placed in the operator's mouth, it was connected to the end of the tube through which the insects are drawn into the exhaustor. This ensured that the indrawn insect could not enter the main part of the exhaustor direct and remained in the bend of the lower tube, from which it could then be blown out into another isolator on a further test plant without the apparatus being taken apart. In these tests, as in further experiments from which information was obtained about the course of virus symptoms, young seedlings of the following varieties were used as test plants: Oats Valečovský bílý, wheat Ratbořská jarní and Blanka jarní, barley Stupický plnozrnný and Triumf, rye Těšovské jarní.

Determination of the period essential for the vector to stay on the plant in order to bring about infection was carried out with naturally infected imagos of *C. pellucida* from the oats epidemic area. The leafhoppers were put individually to feed on the test plants at graded intervals of time. The experiment was carried out twice during June and July 1958. Results are recorded in Table II.

In experiments involving the mechanical transmission of WSV inocula prepared from diseased plants and from infected leafhoppers were used. Preparation of the inoculum from plants was carried out as follows: Leaves picked from typically striated wheat plants were cut up into small pieces and 5 g. of this material was put into a cooled porcelain mortar. Following the addition of 10 ml. of cold phosphate buffer (Sörensen) of pH 7 and a pinch of sand, the mixture was thoroughly ground. Liquid was squeezed out of the pulp through a Silon sheet into a measuring glass and the squeezed pulp was returned to the mortar, where it was mixed with a further 5 ml. of buffer and ground again. The homogenate was put into the Silon sheet and thoroughly squeezed again by hand and by means of virological forceps, the expressed liquid being added to that in the measuring glass. In this way 16·1 ml. of inoculum was prepared. Inoculation followed at once. It was carried out by transferring the inoculum by means of a pipette into small swabs of folded gauze, which were sprinkled with carborundum powder; the leaves of the young test plants (60 oats, 30 wheat and 30 barley, sown 18. 6. 1957 in 10 cm. jars, 5 in each jar, inoculation carried out 3. 7. 1957 at the phase of the 1st to 3rd leaves) were lightly wiped several times with these gauze swabs. The methods used in the experiments on the mechanical transmission of cereal viruses using inoculum obtained by extracting homogenates from the bodies of infected leafhoppers have been published in a previous paper (Průša et al. 1959).

The attempt to transmit cereal viruses through the soil was started in May 1958 on a fenced plot in the garden. The fenced plot was filled to about 10 cm. with a layer of soil into which in the previous autumn a considerable amount of chopped straw and roots from oats plants suffering from OSDV, and wheat infested with WSV, had been placed. Four rows of oats and one row of wheat were sown in this plot. The possibility of infection from without was eliminated by placing a large, angular isolation cage of Silon fabric with a wooden frame over the whole plot. A total of 406 oats (Valečovský bílý) and 102 wheat (Blanka) plants were included in the test.

The thermal inactivation point of cereal viruses, which were found to be untransmittable by mechanical means, was examined directly within the bodies of live leafhoppers. Naturally infected insects, caught on crops in the area where disease had developed, were placed fifteen at a time in thin-walled test-tubes closed by fine silon fabric. The test-tubes were put into a water bath for varying periods of time. The temperature of the water baths was maintained at exactly 35° C, 37·5° C and 40° C by means of Vertex and electronic contactors (apparatus shown in Fig. 2). These groups of leafhoppers were then placed on healthy oats plants in order to test their infectivity.

Results and Discussion

Experiments with Vectors

As shown in Table I, in no part of the first experiment were plants with typical signs of WSV observed. Plants covered with aphids and bugs remained almost entirely healthy. Only in the variant using leafhoppers were 15 out of 75 test plants found to be infected with OSDV. OSDV was transmitted in this case from the striated wheat, which was evidently simultaneously infected with OSDV, although this could not be recognised from the symptoms.

In addition, it was recorded that some plants, in which aphids had been used for transmission, developed small, brownish or rusty-yellow spots about 10 days after the feeding (Fig. 3). These were mostly to be found on the oldest leaf and it was usually possible to see a minute, dark-

Table 1. Attempt at transmitting WSV by three groups of insects (1957)

Feeding period		Species mixture	No. of insects on plant	No. of test plants	Date of sowing oats	Diseased	
Acquisition on diseased wheat	Test on healthy oats					WSV	OSDV
						No.	No.
14.—17. 6.	17.—24. 6.	aphids	3—5	35	5. 6.	0	0
				40	11. 6.	0	0
14.—17. 6.	17.—24. 6.	bugs	2—3	35	5. 6.	0	0
				40	11. 6.	0	0
14.—17. 6.	17.—24. 6.	leafhoppers	2—3	35	5. 6.	0	7
				40	11. 6.	0	8
Non-infected control plants:				70	5. 6.	0	0
				80	11. 6.	0	0

coloured centre indicating the point of puncture. After feeding by bugs, spots of a different character appeared on the leaves of young plants; they were more numerous and looked like pale dots, while as in the above case they were still to be seen a long time after the killing of the tested insects. Sporadically some of both kinds of spots grew considerably larger, took on an ochre-brown colour and acquired a watery consistency. Since it was demonstrated, however, that none of these spots, which were met with here and there in later experiments with sucking insects as well, had any causal connection with WSV or OSDV, no great attention was devoted to them.

The series of tests carried out concurrently with aphids collected from crops and grasses in the area of virus diseases gave almost entirely negative results and therefore no further description has been given here. These tests, however, confirmed that aphids have no connection with the spreading of WSV.

During February and March 1958 a pilot experiment with 12 leafhoppers *(C. pellucida)* was carried out under laboratory conditions. They were adult females whose development had been artificially accelerated by adjustment of the temperature and lighting conditions. They came from the OSDV epidemic areas, having been caught as larvae in the autumn of the preceding year. The insects were placed individually on 6 oats and 6 wheat plants. In each case 3 leafhoppers were left on each host plant for 20 hours, another 3 for 3 days. The insects were then removed and the experimental plants were given supplementary illumination.

None of the plants showed any of the characteristic signs of WSV. OSDV developed, however, in one oats and one wheat plant. In both cases this took place in the variant with the 20-hour test feeding period. The rest of the plants remained healthy. This very limited experiment showed for the first time the fundamental differences in pathogenic capacity between individual leafhoppers of the same sex, age and origin, for only 2 out of 12 produced typical signs of OSDV after less than a day on the host, while others, evidently uninfected, did not produce this result even after 3 days of feeding. In addition, this result already indicated quite clearly that the virus of wheat striation is probably not identical with OSDV, because in the case of the only successfully infected wheat plant the characteristic stripes failed to

develop; on the contrary, the symptoms of disease (dwarfing, more intense tillering, slight yellowing from the tips of the youngest leaves) were those which were later found to be caused by OSDV (Průša 1958, Průša et al. 1959).

At the beginning of April a more extensive experiment of five months duration was started in order to investigate the pathogenic effects of 160 leafhoppers *C. pellucida* from Sázava (locality where the occurrence of WSV and OSDV was greatest) on various cereals. Each leafhopper was tested individually on a certain series of cereals. The periods of test feeding ranged from one to three weeks in most cases. The leafhoppers were tested from the stage of hibernating larvae throughout the whole of their further development, usually up to their natural death. As a rule they fed on 6 to 9 test plants consecutively. For the first three passages larvae of the third to fifth stage of growth were used, while from the end of the fourth passage, by which time many insects had already matured, adults were used. The results obtained enabled the leafhoppers to be divided, according to the pathological state which developed following their feeding on the test plants, into the following four groups:

1. Non-pathogennic insects. There were 23 leafhoppers in this group (14·4%). Plants on which they fed were never diseased. Following their feeding either no slowing of growth took place, or there was a slight slowing which was atypical as regards the disease concerned and became somewhat more pronounced with increasing length of the insect's stay on the host plant.

2. Insects calling forth symptoms typical of OSDV. There were 96 leafhoppers in this group (61·2%).

3. Insects producing symptoms characteristic exclusively of WSV. There were only 3 leafhoppers in this group (1·9%).

4. Insects causing for the most part symptoms typical of OSDV, but occasionally of WSV as well, or of both viruses simultaneously. A group of 36 insects behaved in this way (22·5%).

Further, it was found that the leafhoppers tested transmitted OSDV not only in a high percentage of cases (83·7%), but also very regularly and usually to all plants on which they had the opportunity to feed. As opposed to this WSV was not transmitted nearly so frequently and regularly, but only at irregular intervals of time and usually to a minority of the test plants. Among a total of 39 leafhoppers (24·4% of the total) infected with WSV there were remarkable differences in the efficiency of transmission of this virus. Only 3 individuals (7.7%) infected all the plants on which they fed, 10 leafhoppers (25·6%) infected a half to three-quarters of the plants and the remaining 26 leafhoppers (66·7%) infected less than half the plants on which they were placed. While no particular differences were found in the efficiency with which *C. pellucida* transmits OSDV, the facts noted above indicate that there exist races with varying efficiency as regards their ability to transmit WSV. It would appear that in our field populations the less efficient races predominate. This assumption is supported by field observation according to which WSV occurs only sporadically in the crops, which would otherwise be difficult to explain.

The finding of considerable differences among individual leafhoppers as regards their efficiency in transmitting WSV is in complete agreement with a similar observation on the European cereal striate mosaic virus, which was published recently in Great Britain (Watson 1959, Watson and Sinha 1959). This virus is probably identical with our WSV. The above authors

distinguished three races of the vector according to the ability to acquire and transmit the virus: highly efficient (transmitting the virus to 90 to 100% of the test plants), efficient (transmitting to 40 to 50% of the test plants), and inefficient (transmitting to 10 to 20% of the test plants). Although more accurate data on the representation of the different races in field populations have not yet been obtained, it is assumed that in England, too, the least efficient race predominates.

Dlabola (1958a, 1958b), who worked with similar material to that used by the present authors, mentions that he observed signs of striation only after feeding by *C. pellucida* larvae placed in large numbers on the cereal crops. He considered the cause to be due rather to some kind of toxin injected by the leafhopper with its saliva into the plant during feeding than to a virus. In the present tests, on the contrary, in spite of a certain irregularity and fluctuation in transmission (the maximum transmission of WSV was for example recorded following test feeding at the end of April and the beginning of May, when the majority of leafhoppers were in the last but one and last phase before transformation to imagos), it was definitely established that ability to transmit WSV is not limited to any special stage of development of the vector, but that it is exhibited by larvae at different stages and by imagos of both sexes. Further, one leafhopper suffices for infection.

Finally, the above experiment also showed that WSV persists in its vector for a very long time (e.g. one leafhopper transmitted WSV to 6 plants over a period of several months) and that it therefore belongs to the so-called persistent viruses.

The Relation between the Length of the Vector's Stay on the Host Plant and the Probability of Successful Infection

The results summarised in Table II show that the first transmission of OSDV took place after a thirty-minute stay by a leafhopper on its host, while in the case of WSV nine hours were necessary.

Table 2. Examination of the period of feeding by the leafhopper *Calligypona pellucida* on oats plants necessary to cause infection

Length of stay of a leafhopper on host	Tested leafhoppers	No. of leafhoppers transmitting OSDV		No. of leafhoppers transmitting WSV	
		total	%	total	%
5 minutes	21	0	0	0	0
10 minutes	20	0	0	0	0
30 minutes	20	1	5	0	0
60 minutes	20	2	10	0	0
90 minutes	20	2	10	0	0
2 hours	20	3	15	0	0
3 hours	20	3	15	0	0
6 hours	20	5	25	0	0
9 hours	20	7	35	1	5
15 hours	10	4	40	1	10
20 hours	20	9	45	1	5
24 hours	20	11	55	2	10
30 hours	14	8	57·1	2	14·3
2 days	16	10	62·5	2	12·5
3 days	9	9	100	1	11·1
4 days	8	8	100	1	12·5
6 days	6	6	100	2	33·3

It must be noted, however, that irregularity in WSV transmission, due to the probable existence of vector races of varying efficiency, and the fact that there were relatively few individuals infected with this virus among the leafhoppers used, makes it necessary to assume that a shorter feeding period could suffice to produce infection by WSV, too. This view is also supported by data from England (Slykhuis and Watson 1958), according to which it was observed that European cereal striate mosaic virus was exceptionally transmitted after only half-an-hour of test feeding.

The experiment also showed that the duration of test feeding by the vector has an effect within a certain time interval on the probability of transmission of the disease, but not on its destructivity. The plants exhibited practically equally severe signs of disease when they were infected following feeding by the infected insects lasting several hours or some days.

Attempts at Mechanical Transmission of WSV

The first attempt at mechanical transmission of WSV using sap from striated wheat was evaluated at the end of August 1957. Out of 120 inoculated plants only one wheat plant showed signs of WSV. All the rest (60 oats plants, 30 barley and 29 wheat) remained healthy. This experiment was later repeated twice without any of the characteristic symptoms of the disease being produced. It was therefore assumed that the single case of transmission was probably accidental and that WSV, like OSDV, is non-transmittable by mechanical means using the sap of diseased plants.

In 1958 an experiment was also carried out to determine whether the two cereal viruses identified in Czechoslovakia can be transmitted by mechanical inoculation (rubbing into stalks and leaves, injection into stalks) with concentrated extracts of the crushed bodies of infected leafhoppers. The negative result of this experiment, which has already been published (Růša et al. 1959), also goes to confirm that WSV and OSDV cannot be transmitted mechanically.

Attempt to Transmit WSV through Dodder (*Cuscuta campestris* Yunck.)

The attempt to transmit WSV by means of *Cuscuta campestris* Yunck. failed. Since, however, the experiment was not sufficiently extensive (only 8 young wheat plants were used for infection), and since the dodder attached itself to the wheat with some difficulty, this result cannot be regarded as final.

Attempt at Transmitting Cereal Viruses through the Soil

None of the 406 oats and 102 wheat plants, grown in soil infested in the autumn of the previous year with considerable quantities of straw and roots from virus-infected cereal crops, showed any symptoms of WSV or OSDV disease. It can thus be concluded that neither of these viruses is transmitted by soil. The result of the experiment is also in agreement with observations made in previous years under field conditions with less strict control (viz Růša 1958).

Attempt at Thermal Inactivation of Cereal Viruses

Since both WSV and OSDV are not mechanically transmittable by sap extracted either from diseased plants or from infected leafhoppers, an attempt was made to establish the point of thermic inactivation of these viruses directly in the bodies of the living vectors. Significant inactivation was not obtained for either virus by warming the leafhoppers to a temperature of 35° C for a period of 3 hours or less, to 37·5° C for 45 min. or less nor to 40° C for 10 min. Following 45 min. heating to 37·5° C, however, more than half the leafhoppers died and following 10 min. at 40° C only one insect out of 15 remained alive.

Histologico-Cytological and Biochemical Examination

Wheat, oats and barley plants infected with both OSDV and WSV were subjected to careful histologico-cytological examination by several reproductions of the method which in the U.S.S.R. enabled specific and diagnostically important phenomena (inclusion bodies) in cereals suffering from the "zukuklivanie" virus and the Russian winter wheat mosaic virus to be demonstrated (Sukhov 1940, 1943, 1956, Sukhov, Vovk and Alexeyeva 1943). In certain respects these viruses resemble those dealt with in the present paper. It was found that in cases of WSV and OSDV disease neither needle crystals nor large looped inclusions appear in the live epidermal and guard cells. These are characteristic in cases of "zakuklivanie" virus disease in cereals. It was further found that, in contrast to the two Russian viruses, in cases of WSV and OSDV

needle-shaped bodies are not even formed after treatment of the sections or tissue homogenates with 0·1 N HCl solution and adjustment of the pH of the medium to 4·0.

The absence of these remarkable intracellular bodies was later kindly confirmed by the leading Soviet virologist V. L. Ryzhkov (1959), who received specimens of WSV and OSDV infected cereals, preserved in alcohol, for investigation.

Further evidence of the distinct character of WSV and OSDV as compared with the Russian viruses was obtained during experiments on crystallizing out of virus substances by methods described in the U.S.S.R. (Sukhov 1943, 1956, Sukhov, Vovk and Alexeyeva 1943). The procedure by which it was possible to prepare the Russian viruses in crystalline form was repeated several times on the present material with negative results.

Symptomatology of Wheat Striate Virus Disease

Concurrently with investigation of the etiology of signs of disease in cereals thorough symptomatological observations were made on oats, wheat and barley which had been naturally, or especially artificially, infected with OSDV and with WSV. It was revealed that WSV differs considerably in its effects on the host from OSDV and that it has a somewhat shorter incubation period.

The first perceptible symptom following WSV infection is not a change in growth, as with OSDV, but a colour change. Three weeks following infection (and later too) striation can appear on the youngest leaves in the form of pale or yellowish lines of varying length. There is an increase in the number of chlorotic lines running parallel to the veins, they lengthen, grow broader and run together to form stripes. This chlorosis passes over to some of the older leaves and to all leaves developing after infection; it may cover the leaf sheaths and parts of the stalks. At a more advanced stage of this typical systemic disease almost the whole of the plant may, therefore, take on a cream colour. The characteristic striation, which is similar on the different cereal species, is illustrated in Figs. 4 and 5.

Following the well-defined appearance of striation the growth of the diseased plants is practically halted. Stimulation of tillering and proliferation in the flower organs, insofar as they develop at all, do not take place. Ears or panicles (glumes are also striated — see Fig. 6) can only be formed in plants that have been infected at a later stage of development; the seeds are then shrunken and inferior.

Plants which have been infected at an early stage (the phase of the first to third leaves) lose their chlorophyll within 6 to 8 weeks and die. Similarly, the majority of cereals infected during the first two months of growth die prematurely. WSV is more destructive of barley and wheat than OSDV. In some cases it was even noted that elongated necroses developed on the chlorotic parts of barley leaves, var. Triumf (Fig. 7). Similar necroses also accompany American wheat striate mosaic virus disease on the wheat variety Minter (Slykhuis 1952, 1953). With oats there was not such a difference in the destructivity of WSV and OSDV, but plants infected with WSV died sooner.

During combined infection by WSV and OSDV the course of the disease is the most severe and death of the hosts takes place most rapidly. Combined infection was most easily recognisable from the external symptoms in oats. Here, in addition to the striation indicating WSV, there quite often appeared symptoms revealing the presence of OSDV (swelling of stalks in the region of the tillering nodes, increased tillering, darker colouring of the green parts, etc.). With

wheat or barley showing symptoms of WSV it was, on the contrary, impossible to decide by the external symptoms whether plants were or were not suffering at the same time from the less destructive OSDV. This could be determined, however, not only from the results of the feedings which preceded and followed in the series of passages of the leafhopper concerned to other test plants, but also from some experiments in which OSDV was transmitted from plants exhibiting symptoms of WSV alone.

Symptomatologically WSV is related to the wheat striate mosaic virus described in the U.S.A by SLYKHUIS (1952, 1953). The American virus is, however, transmitted by the leafhopper *Endria inimica* Say., which does not occur in Europe. A virus which is probably completely identical with WSV has also been described in England under the name European cereal (wheat) striate mosaic virus (SLYKHUIS 1958, SLYKHUIS and WATSON 1958). The present authors do not fully agree with the above authors as regards the naming of the virus, mainly because its characters definitely place it among the so-called "cicadophile yellows" and it should therefore not be termed a mosaic. Examples of striation in cereals observed by SLYKHUIS (1958) during his visit to Germany and Denmark evidently belong to this virus too. WATSON (1959) demonstrated a disease similar to the English virus disease in material brought to England from Spain, where it seems to be attaining dangerous proportions. News of the occurrence of a virus similar to WSV has also been published recently in Sweden (LINDSTEN 1959). All this indicates that WSV is fairly widely distributed in Europe.

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Вирус полосатости пшеницы

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Резюме

В 1954—1956 гг. эпидемически распространилось на Чешско-моравской возвышенности новое заболевание зерновых культур; кроме основного фактора – вируса стерильности и карликовости овса (ВСКО)-играл здесь меньшую роль и вирус полосатости пшеницы (ВПП). Симптоматологическое изучение показало, что ВПП на зерновых — хозяевах выявляет характеристические отличия от ВСКО. Однако, ВПП, также как и ВСКО, распространяется специфически цикадкой *Calligypona pellucida* Fabr. Использованием долговременного теста векторов происходящих из местностей зараженных ВСКО и ВПП удалось нам выявить четыре по своему патогенному действии отличающиеся группы насекомых: 1) непатогенные, 2) индуцирующие признаки ВСКО, 3) индуцирующие признаки ВПП, 4) индуцирующие преимущественно признаки ВСКО, однако, иногда также признаки ВПП или обеих вирусов одновременно. ВПП персистирует в своем хозяине и переносится как личинками разных фаз развития, так и имаго *C. pellucida* обеих полов. ВПП по сравнению с ВСКО переносится менее регулярно. Между отдельными цикадками наблюдаются значительные различия в степени эффективного переноса ими ВПП, следовательно, возможно предполагать существование в этом направлении в разной степени эффективных рас. В полевых популяциях у нас преобладают менее действенные расы. Для успешного переноса ВПП надо по меньшей мере девяти часов пребывания вектора на хозяине, однако кажется, что достаточно и более короткого срока. Экстракты из разрушенных листьев полосатых пшениц, также как и из инфекционных цикадок, инокулированные в растения механически (втиранием, инъекцией) не привели к заражению. Попытки перенести вирус посредством почвы или повилики (*Cuscuta campestris* Yunck.) не увенчались успехом. Точку термической инактивации в живом переносчике установить не удалось. В СССР описанные вирусы закукливание злаков и русская мозаика озимой пшеницы отличаются от ВСКО и ВПП, кроме другого, также и продукцией типичных включений и тем, что их легче возможно привести к кристаллизации.

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The Size of Pollen Grain of the Genus *Triticum* L.

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Souhrn

1. Úkolem práce bylo prostudovat velikost pylových zrn u jednotlivých druhů polyploidní řady r. *Triticum* L.

2. Velikost pylových zrn jsem zjišťoval u 15 vzorků patřících do 8 druhů. Měření jsem prováděl na trvalých acetokarmínových preparátech.

3. Velikost pylových zrn u různých druhů r. *Triticum* stoupá v souhlasu s počtem chromosomů. Pro jednotlivé skupiny byly vypočteny tyto průměrné objemy: pro diploidní skupinu 62.10^3 μ^3, pro tetraploidní skupinu 96.10^3 μ^3 (z toho pro *T. timopheevi* Zhuk. 73.10^3 μ^3) a pro hexaploidní skupinu 118.10^3 μ^3.

4. Objem pylových zrn není tedy příslušným násobkem počtu chromosomů. Tento nesouhlas s teoretickým předpokladem o přímé lineární závislosti mezi počtem chromosomů a velikostí pylových zrn je patrně podmíněn allopolyploidií v polyploidní řadě r. *Triticum*. Zdá se, že geneticky rozdílné genomy A, B, D a G mají také rozdílný účinek na velikost pylového zrna.

5. Výpočty ukazují, že genom B má 62 %, genom D 29 % a genom G 18 % účinku genomu A (100 %). Podle působení na objem pylového zrna tvoří tedy tyto genomy řadu A > B > D > G.

Summary

1. The aim of the present paper was an investigation of pollen grain size of different species of the *Triticum* L. natural polyploid series.
2. We determined the pollen grain size of 15 specimens belonging to 8 species. The determination was carried out on permanent acetocarmine preparations.
3. The pollen grain size of different *Triticum* species tends to grow with the increasing number of chromosomes. The following mean volumes were computed for the different groups: 62.10^3 μ^3 for the diploid group, 96.10^3 μ^3 for the tetraploid group (of which 73.10^3 μ^3 for *T. timopheevi* Zhuk.), and 118.10^3 μ^3 for the hexaploid group.

4. As can be seen, the volume of the pollen grain does not represent a corresponding multiple of the chromosome number. The reason for this disagreement with the theoretically expected linear relation between the chromosome number and the pollen grain size lies apparently in the allopolyploidy of the polyploid series of the genus *Triticum*. It seems that the genetically different genoms A, B, D, and G also exert a different effect on the pollen grain size.
5. Our calculations show genom B to have 62%, genom D 29%, and genom G 18% of the effect of genom A (100%). These genoms form a sequence A > B > D > G with respect to their effect on pollen grain volume.

Introduction

The species of the genus *Triticum* form a natural polyploid series which comprises diploid, tetraploid, and hexaploid species (Sakamura, 1918) if we do not consider some amphidiploid interspecific hybrids which are octoploid and decaploid (Zhebrak, 1957). It is well known that the multiplication of the chromosome number usually causes the enlargement of nuclei and whole cells (Lindstrom, 1936). This enlargement is the most conspicuous with specialized cells, the size of which is usually fairly stable, like pollen grains and stoma guard cells (Stebbins, 1957). Investigations of some plants have shown the volume of these cells to be proportional to the chromosome number. This was, for example, proven by Lindstrom and Humphrey (1933) and Humphrey (1934) for the autopolyploid forms of tomato plants. Firbas suggested the existence of a similar dependence for the polyploid series of wheats (1937).

The author aimed at a more detailed investigation of the pollen grain size of different species of the genus *Triticum*.

Material and Methods

The pollen grain size of 15 specimens of different species of the genus *Triticum* was determined. The group of diploid wheats was represented by the species *T. monococcum* L. var. *hornemanii* Clem., the group of tetraploid wheats by the species *T. timopheevi* Zhuk. var. *typicum* Zhuk., *T. dicoccum* Schrank var. *farrum* Körn. *T. durum* Desf. var. *candicans* Meist. (variety Candicans 76,10), *T. durum* Desf. var. *hordeiforme* Körn. (variety Hordeiforme 10), *T. durum* Desf. var. *melanopus* Alef. (variety Melanopus 69), *T. turgidum* L. var. *salomonis* Körn., *T. persicum* Vav. var. *juliginosum* Zhuk., the group of hexaploid wheats by the species *T. compactum* Host. var. *erinaceum* Körn., *T. vulgare* Vill. var. *caesium* Alef. (variety Caesium 111), *T. vulgare* Vill. var. *milturum* Alef. (variety Milturum 321), *T. vulgare* Vill. var. *milturum* Alef. (variety Podbořanka), *T. vulgare* Vill. var. *lutescens* Alef. (variety Ratbořská), and *T. spelta* L. var. *duhamelianum* Korn. All investigated forms were spring wheats from the collection of the genetical section of the Department of Plant Physiology and Genetics, Brno where they have been in cultivation for many years.

The pollen grain size measurements were carried out on permanent preparations. Directly before the beginning of flowering several ears were cut of the main stems of each investigated

specimen. Three to four ripe but still unopened anthers were put on a slide. As soon as they opened they were removed with a forceps and the shed pollen was evenly distributed in the centre of the slide by knocking at its edge. The required quantity of staining and enclosing liquid was then added (a mixture of a staining acetocarmine solution and liquido Faure 1 : 1), the coverslip was mounted and the slide heated. The resulting preparations were marked, stored, and about one year later measured.

A micrometer ocular which allowed measuring with an accuracy of about 2·5 μ at a 1200-fold magnification was used for the determinations. Three slides of each specimen were prepared. The longer diameter of a random choice of one hundred pollen grains in the slide centre was measured; thus for each specimen the size of a total of 300 pollen grains was determined.

Results

The results of the measuring are given in tab. 1. They show that the pollen grain size increases in agreement with the chromosome number. While in the group of diploid wheats the longer pollen grain diameter was 49·0 μ, it increased to an average of 56·8 μ in the group of tetraploid wheats and to 60·9 μ in the group of hexaploid wheats. The calculation of the volume (on the assumption that we have a sphere round the longer diameter of the pollen grain) gives 62.10^3 μ^3 for the diploid, 96.10^3 μ^3 for the tetraploid, and 118.10^3 μ^3 for the hexaploid groups.

Table 1.

Pollen grain size of different species of the genus *Triticum* L. (Longer diameter in μ)

Species (variety)	$\bar{x} \pm 3 . s_{\bar{x}}$	$\pm s$	v (%)
2n = 14			
T. monococcum L.	49·0 ± 3 . 0·19	± 3·13	5·9
2n = 28			
T. timopheevi Zhuk.	51·9 ± 3 . 0·19	± 3·23	6·2
T. dicoccum Schrank	57·8 ± 3 . 0·18	± 3·18	5·5
T. durum Desf. (Candicans 76/10)	57·2 ± 3 . 0·21	± 3·62	6·3
T. durum Desf. (Hordeiforme 10)	57·9 ± 3 . 0·21	± 3·65	6·3
T. durum Desf. (Melanopus 69)	56·0 ± 3 . 0·20	± 3·53	6·3
T. turgidum L.	59·5 ± 3 . 0·24	± 4·18	6·9
T. persicum Vav.	57·4 ± 3 . 0·20	± 3·47	6·1
2n = 42			
T. compactum Host.	59·9 ± 3 . 0·21	± 3·68	6·2
T. vulgare Vill. (Caesium 111)	59·3 ± 3 . 0·23	± 3·98	6·7
T. vulgare Vill. (Niva)	60·0 ± 3 . 0,17	± 2·90	4·8
T. vulgare Vill. (Milturum 321)	59·0 ± 3 . 0·18	± 3·15	5·3
T. vulgare Vill. (Podbořanka)	61·6 ± 3 . 0·22	± 3·78	6·1
T. vulgare Vill. (Ratbořská)	64·2 ± 3 . 0·20	± 3·50	5·5
T. spelta L.	62·1 ± 3 . 0·24	± 4·15	6·7

Discussion

The above results specify the older data of Firbas (1937) who was the first to suggest that the pollen size increases in proportion to the chromosome number in the polyploid series of wheat. But his paper shows a certain lack of accuracy of measurement (to 4·75 μ), a small number of measured pollen grains (100) and a relatively small number of investigated specimens. Besides, the values computed by Firbas are considerably lower than those found by us and by other authors. This may be caused by the treatment of the pollen in an alkaline medium as was correctly stated by Fjodorova (1956).

The calculations of mean pollen grain volumes in each group show an interesting feature: although the pollen grain size is increases in proportion to the chromosome number, when calculating the volume we find that the mean values for each group do not correspond to the appropriate multiples of the chromosome numbers. Thus, the volume of tetraploid wheat pollen does not increase twice in comparison with the diploid *monococcum* wheat but only 1·6 times; the pollen volume of hexaploid wheat is not 1·5 times that of the tetraploid wheat group but only 1·2 times; also the pollen volume of the hexaploid group does not increase three times as compared with the diploid *monococcum* wheat but only 1·9 times.

These results seem to be in disagreement with the data given by Lindstrom and Humphrey (1933) and Humphrey (1934) for the haploid, diploid, and tetraploid forms of tomato. These authors found (for pollen mother cells, nuclei, and nucleoli at diakinesis) a direct linear proportional dependence between the corresponding volumes and chromosome numbers. Their data apply to a case of autopolyploidy, however, and ours to one of allopolyploidy. According to the summarizing data of Vavilov (1935) diploid wheats possess genom A, the majority of tetraploid wheats genom AB, and hexaploid wheats genoms ABD. The genetic dissimilarity of the various genoms (A, B, D) evidently also manifests itself in the different influence each of them exercises on pollen grain size.

The value found for *T. timopheevi* Zhuk. also supports this assumption. The values of the longer pollen diameter of this species are highly significantly lower than the corresponding values of all the other tetraploid species. When calculating the volume we obtain a value of 73.10^3 μ^3. According to Vavilov (1935) this species possesses genoms AG. Genom G is not only genetically different from genom B of the other tetraploid species but it evidently also has a different influence on the pollen size.

T. turgidum L. constitutes another rather striking anomaly among the tetraploid wheats as far as pollen size is concerned, the values of the longer pollen diameter being almost 3 μ higher than the arithmetical mean for this entire group. This phenomenon might be connected with the forming of a smaller number of chiasmata by hybrids of this species with other tetraploid wheats (Darlington, 1931), a fact giving evidence of certain differences in their genoms.

Proceeding from the hypothesis that the single genoms A, B, D, and G have a different effect on pollen grain size and considering the computed

mean volumes for the separate groups we may easily calculate the relative effects of these genoms. Taking the pollen grain volume in the diploid group (genom A) as 100% the influence of genom B will in comparison with A be only 62%, of genom D only 29% and of genom G even only 18%. In consequence these genoms form a degressive sequence A > B > D > G from the point of view of their influence on the pollen grain volume.

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Размер пыльцевых зерен в роде *Triticum* L.

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Резюме

1. Задачей настоящей работы являлось изучение размера пыльцевых зерен у отдельных видов рода *Triticum* L.

2. Размеры пыльцевых зерен установлены у 15 проб, принадлежащих к 8 видам. Измерения проводились на постоянных ацетокарминовых препаратах.

3. Размеры пыльцевых зерен у разных видов рода *Triticum* L. увеличиваются согласно увеличению числа хромосом. Для отдельных видов высчитаны следующие средние объемы: для диплоидной группы 62.10^3 μ^3, для тетраплоидной группы 96.10^3 (для принадлежащей сюда *Triticum timopheevi* Zhuk. 73.10^3 μ^3) и для гексаплоидной группы 118.10^3 μ^3.

4. Следовательно, объем пыльцевых зерен не является соответствующим кратным умножением числа хромосом. Это несогласие с теоретическим предположением о прямой линеарной зависимости между числом хромосом и размером пыльцевых зерен, повидимому, обусловлено аллополиплоидией в полиплоидном ряду рода *Triticum* L. Кажется, что генетически разные геномы A, B, D, G влияют также по разному на размеры пыльцевых зерен.

5. Расчеты показывают, что геном B обладает 62%, геном D 29% и геном G 18% действия генома A (100%). Следовательно, по степени влияния на размер пыльцевых зерен составляют эти геномы ряд A > B > D > G.

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Photosynthesis in Leaf Disks as a Measure of Photosynthetic Capacity in Crop Plants

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Souhrn

V předcházející práci (Bartoš, Kubín a Šetlík 1960) definovali jsme kapacitu fotosynthesy listů dané rostliny nebo populace rostlin jako intensitu fotosynthesy vzorků z těchto listů ve standardních a pokud možno optimálních podmínkách. Výsledky uvedené v této práci svědčí o tom, že fotosynthesa jednotlivých vzorků z listů rostlin v populaci odrůdy je poměrně málo variabilní hodnota. Proto lze vymezit průměrnou fotosynthetickou kapacitu odrůdy (za daných podmínek pěstování) velmi přesně i na vzorcích z poměrně malého počtu rostlin, např. 12 až 32.

Fotosynthetickou kapacitu jsme měřili metodou přírůstků sušiny v terčících z listů. Využili jsme specifických výhod, které má tato metoda pro srovnávací pokusy a porovnávali jsme fotosynthetickou kapacitu populací různých odrůd kulturních rostlin pěstovaných ve stejných podmínkách. Zjistili jsme, že v některých případech není možno prokázat rozdíl (při $P = 0{,}05$) mezi populacemi různých odrůd téhož druhu v průměrné kapacitě fotosynthesy, jindy jsme nalezli malý průkazný rozdíl. Populace odrůd, náležejících různým druhům, liší se co do fotosynthetické kapacity někdy podstatně, jindy velmi málo.

Největší diference jsme nalezli ve fotosynthetické kapacitě mezi jednotlivými rostlinami v dané populaci, avšak ani ty nepřesahují zpravidla 50 %. Obdobně se liší i jednotlivé listy na rostlině.

Při posuzování výsledků v práci uvedených je důležité si uvědomit, že jsme neměřili fotosynthetickou kapacitu při nasycení světlem. Proti původnímu záměru a proti očekávání zvolená intensita osvětlení ($1{,}8 \cdot 10^5$ erg/cm^2 sec) nestačí na nasycení fotosynthesy terčíků asimilačního pletiva; závislost jejich fotosynthesy na intensitě světla má zřejmě poněkud jiný charakter, nežli se běžně udává pro listy vyšších rostlin.

Summary

In a previous communication (Bartoš, Kubín and Šetlík 1960) a method was reported, which makes it possible to measure the average rate of photosynthesis in a representative sample of a large number of leaves. The sampling is made by cutting out disks of leaf tissue, following a definite pattern. Isolated leaf disks are then illuminated under controlled conditions and the rate of photosynthesis is estimated by weighing the increment of dry matter in a given time interval.

Data published in the present paper demonstrate that this method can be successfully used for a characterisation of photosynthetic capacity (specific rate of photosynthesis, see Bartoš, Kubín and Šetlík 1960). Both specific values for individual plants and average values for homogenous plant populations may be estimated with very good accuracy. One of the chief factors which render this possible is obviously the surprising homogeneity of the photosynthetic tissue of leaves. Samples of very small size may serve as representative of a large total leaf area, e.g. of a population under field conditions.

Preliminary results of comparative investigations suggest that, with the plant varieties examined, differences in photosynthetic capacity are not very great, both between and within populations of diverse varieties. Differences as large as 50% have been encountered so far solely between values of photosynthetic capacity estimated for individual plants in a population. On the other hand, values of average photosynthetic capacity in populations of crop plant varieties within one species or genus are, as a rule, not very distinct and quite often no statistically significant difference can be demonstrated in such cases. Even if the varieties belong to different genera, differences in photosynthetic capacity in their populations greater than some 50% have been found only as an exception.

In considering all these results one important fact must be taken into account, i.e. the intensity of illumination in the apparatus. Contrary to what was intended and awaited, the radiation intensity used was not high enough to saturate photosynthesis in leaf disks. The light dependence of photosynthesis in isolated portions of leaf tissue apparently has a somewhat different character from that usually assumed for intact leaves of higher plants.

Introduction

The effort to find a reliable comparison of the specific photosynthetic efficiency (capacity) of various plant species and varieties has for a long time been one of the fundamental currents in photosynthesis research. This is quite understandable in view of the importance which might be ascribed to proved significant hereditary differences in the specific rate of photosynthesis in plant breeding and other important questions of agriculture. Numerous papers have dealt with this question (e.g. Boysen-Jensen 1931, Tageeva 1932, Larsen 1942, Wassink 1946, Asana and Mani 1950, 1955, Blackman and Wilson 1951, Watson 1952, Begishev 1953, Nichiporovich 1954) and diverse methods have been tried to solve the problem. So far, however, none of the procedures adopted has proved completely successful. As a matter of fact, an unequivocal proof that taxonomic specificity of photosynthesis exists, has not been given hitherto.

Recently, we described a method (Bartoš, Kubín and Šetlík 1960) which makes it possible to measure the average rate of photosynthesis in a large number of leaf samples simultaneously and under standard conditions. The nature of the method allows for reliable comparison of photosynthetic capacity in samples of different origin and the present paper brings the first data from comparative measurements on a limited number of crop plant varieties. The results, which may be drawn from these data, are summarised in the introductory paragraph. A more detailed account is given further, from which better orientation may be acquired as to the variability of photosynthetic capacity in different types of crop plants.

Material, Methods and Techniques

Experimental plants were grown under conditions specified in the previous communication (Bartoš, Kubín and Šetlík 1960). Since comparative measurements were intended, individual varieties were grown on replicate plots interspersed according to the usual practice for field experiments. When an average sample was collected, leaves were sampled from randomly selected plants on all replicates.

Seedlings of fodder cabbage and kohlrabi plants were planted 40 × 40 cm. apart; sunflower was sown in different stand densities (50 × 50 cm., 25 × 25 cm. and 12·5 × 12·5 cm.), corn in rows 70 cm. apart and with average spaces of 50 cm. along the row. Leaves of cucumber and pumpkin plants were collected on a neighbouring field from plots grown for another purpose. Thus, the conditions under which these plants were grown, were not identical to those mentioned above.

In the following text individual varieties are referred to either by an abbreviation or simply by a figure. Correct names of the sorts are as follows:

Correct name of the variety	Abbreviation used	Reference figure
Brassica oleracea var. *gongylodes* L.		
kohlrabi		
var. Pražská bílá	early kohlrabi	1
var. Wiener weisser	late kohlrabi	2
Brassica oleracea var. *acephala* DC.		
fodder cabbage		
var. Coulet de Flandre	red fodder cabbage	3
var. Markstammkohl	green fodder cabbage	4
Zea mays L.		
corn		
var. Trebišovská raná	early corn	5
var. Bučianský koňský zub	late corn	6
Helianthus annuus L.		
sunflower		
var. Slovenská sivá	tall sunflower	7
var. NS 147 (breeding material from the station Sládkovičovo)	low sunflower	8

Cucumis sativus L.

cucumber
a homogeneous population of a unknown variety 9

Cucurbita pepo L.

pumpkin
six plants of an unknown variety 10

Samples from leaves of red fodder cabbage were used in a large number of experiments. Its thick and stiff leaves, large as they are and with relatively large areas practically devoid of veins offer a very suitable material for measurements performed by a technique in which maximal homogeneity of samples represented by sets of leaf disks is critical. Besides, with this plant variety, leaves of very similar character and individual stage of development are available for the greater part of the growing season. It is, therefore, in a certain sense possible to relate all the measurements throughout this work to the nearest measurement taking red fodder cabbage as a kind of standard material.

The procedure of sampling material and performing the experiment was the same as in the foregoing paper, where it was described in detail. The detailed specification of the procedure for individual experimental series referred to in this paper is synoptically presented in Table 1, where a short characteristic of the chief aim pursued in separate experiments is found in the last column.

As illustrated by a separate experiment, the duration of storage of the detached leaves in the dark before the experiment proper is likely to influence the results with certain plants. It will, therefore, be necessary to examin this point thoroughly in order to find truly standard conditions for comparison of different varieties as to their capacity for photosynthesis.

Results

The chief aim of the work described in this paper was to decide if a reproducible value, which would be characteristic for photosynthetic capacity in leaves of individual plants and of average photosynthetic capacity in leaves of crop plant stands (populations), is in any way accessible to exact measurement.

Measurements were performed by the dry-weight increase method with representative samples of leaf tissue, collected following a definite pattern. The results of measurements presented here demonstrate that the aim marked out above may be arrived at and that comparisons of photosynthetic capacity, as one of the physiological characteristics, are possible at least with the given populations and for the specific set of conditions in which they are growing.

It was further of great interest to estimate the minimal size of the representative sample which is necessary for reproducible values to be obtained for plant populations. This will be determined by the minimal number of plants and the minimal area of the individual subsamples taken from one plant, fulfilling the condition that two analogous samples of this size, selected at random in the given population yield corresponding results. As to the criterion of identity, it was our opinion that only statistically significant differences in the rate of photosynthesis over $\pm$ 5% should be considered as important. In other words, samples yielding results within $\pm$ 5% were not considered as different in their photosynthetic capacity.

It has been found that samples which have a surprisingly small area in comparison with the leaf area of the plant and samples including a relatively small number of plants from a given population may meet the conditions postulated above. It thus seems possible, with a reasonable expenditure of labour, to compare the photosynthetic capacity of individual plants as well as different populations.

Table 1

Main object of the experiment	Comparison of different varieties	Comparison of different varieties	Comparison of different varieties	Comparison of individual plants and leaves	Temperature dependence	Influence of the storage time	Reproducebility of results	Influence of light intensity
No. of experimental series	3–58	5–58	6–58	7–58	11–58	12–58	23–58	24–58
No. of exposures (runs) in the series	4	4	4	4	4	4	2	2
Varieties examined (the figures explained in text)	1, 2, 3, 4	3, 4, 5, 6	7, 8, 9, 10	7	[6] 3, 5, 8, 9	[8] 3, 7, 3*, 7*	[10] 3+3*	[11] 3, 3*
No. of individual plants belonging to one variety which were examined in the series	32	24	32	4	24	12+12*+ *12+*12*	24+24*	24
No. of individual plants belonging to one variety which were included in one run	8	6	8	[4]	6	3+3*+ +*3+ *3*	12+12*	12
No. of leaves sampled on one plant 2	3	3	3	4	3	3	3	3
Number/diameter in mm.) of disks sampled from one leaf	8/8	6/12	8/8	[5] 6×18/8	6/8	6/8	6/8	6+6*/8

Date of sampling (detaching) the leaves	26. a 27. 7.	1. 2. a 3. 8.	8. 9. a 10. 8.	13. a 14. 8.	20. 8.	27 a 29*. 8.	9 a 10. 10.	15. 10.
Date of the measurement	26. 27. 28 a 29. 7.	1. 2. a 3. 8.	8. 9. a 10. 8.	13. a 14. 8.	20 a 21. 8.	29. 8.	9 a 10. 10.	15. 10.
Time in hours of storing leaves in the dark	24 a 48	12 a 24	6, 12 a 18	6, 10 a 18	12, 16, 20, 24	3* a 48	4	4 a 10
The number of columns and rows of openings in the matrix [1]	24 × 32	18 × 24	24 × 32	18 × 24	18 × 24	18 × 24	18 × 24	18 × 24
Sequence of samples from different varieties in the columns of the matrix	8 × [3] 1–2–3–4–....	6 × 3–4–5–6–...	8 × 7–8–9–10–..	[4]	6 × [7] 3–5–8–9–...	3 × 3–7–3*–7*– –*3–*7– –*3*–*7*	12 × 3–3*–	12 × [12] 3a–3b–3c.... 12 × 3a*–3b*– -3c*...
Air temperature in the chamber average temperature of the disks	25° C/27·5° C	24° C/26° C	25° C/27·5° C	22·5° C/25° C	19·5°C/24°C 21·5°C/26°C 28°C/30 C 32°C/34°C	22·5° C/25° C	21·5° C/ /23·5° C	22·5° C/25° C
CO_2 – concentration in the circulating atmosphere	0·5%	0·5%	0·5%	0·2%	0·3%	0·5%	0·3%	0·3%
Irradition intensity erg/ /cm². sec	$1{\cdot}5 \cdot 10^5$	$1{\cdot}8 \cdot 10^5$	$1{\cdot}8 \cdot 10^5$	$1{\cdot}8 \cdot 10^5$	$1{\cdot}8 \cdot 10^5$	$1{\cdot}8 \cdot 10^5$	$1{\cdot}8 \cdot 10^5$	$1{\cdot}8 \cdot 10^5$
Time in hours of one exposure	8	6	3	6	3 a	3 a *6	3 a 6*	4

Table 2. Dry weight increments of individual samples in mg. per 9 cm². (this is the total area of one separately weighed set of 18 disks with 8 mm. diameter). Data from experimental series No. 23-58, material: red fodder cabbage. For the exposure of three hours the primary data are presented, with the 6-hour exposure the actual values of dry weight increments have been divided by two in order to facilitate direct comparison

No. of experiment	Exposure time	No. of group (see Table 4)			
		2	3	4	1
23-1-58	3 hours	8·3	10·8	10·1	9·0
		12·5	9·2	11·3	10·2
		11·2	11·1	9·8	10·2
	6 hours	8·9	11·8	10·7	9·7
		10·9	9·6	9·1	10·9
		9·8	10·7	12·0	9·8
23-2-58	3 hours	10·6	9·6	12·2	10·3
		11·2	11·5	10·8	12·5
		11·8	11·9	9·7	11·7
	6 hours	10·7	10·6	12·4	11·3
		10·6	10·5	10·5	10·6
		11·8	10·6	10·8	9·5

The data of experiments 23-1,2-58 presented in Table 2 may be considered as typical with respect to the variability of photosynthetic capacity in leaf samples from plants within one population. Average values based on 12 separate samples each for two runs (3 and 6 hours) of the same experiment agree well in both cases (expt. 23-1 and 23-2). The increments of dry

Table 1.

[1] The number of openings in one column (first figure) gives the number of disks in one separately weighed sample. The number of openings in one row (second figure) gives the number of individual samples. In most experiments one weighed sample represents one plant.
[2] The leaves were usually selected so as to represent an average sample along the stem.
[3] Read: the given succession of varieties in columns (1—2—3—4) repeated eight times periodically. The figures refer to individual varieties as explained in the text.
[4] The second, fourth, sixth and eighth leaves on the stem were sampled from four solitary sunflower plants. In successive runs samples from all leaves of two plants were always combined so that a cross-control was ensured.
[5] One individual leaf was represented by six sets of disks, giving six separately weighed samples with 18 disks each.
[6] The samples from plants of one variety tested in different temperatures originated from the same plants as samples in experiments 5-58 an 6-58.
[7] One pair of values refers to each of the four exposures.
[8] The asterisk to the right of the figure indicates leaves stored 3 hours in the dark, asterisk to the left leaves stored 6 hours.
[9] Indexes indicate plants from different stand densities: a — solitary plants, b — stand density 50 × 50 cm., c — 25 × 25 cm. and d — 12·5 × 12·5 cm.
[10] The asterisk to the right of the figures indicates samples with six hours exposure.
[11] The asterisk indicates samples illuminated by lower intensity.
[12] From each plant two parallel samples were taken (disks cut in close vicinity) one for each of the two intensities.

Table 3. Average values of dry weight increments in isolated leaf disks from red fodder cabbage calculated for individual runs in experiments 23-1,2-58. Results expressed as mg. d. wt. increase per dm^2. per hour

No. of experiment	Exposure time in hours	3	6	Average
23-1-58	$\bar{d} \pm t_{0.05} \cdot s_d$ mg./$dm.^2$ hod.	37·93 ± 2·68	38·20 ± 2·36	38·06 ± 1·68
	V	17·87	13·75	15·82
	n	12	12	24
23-2-58	$\bar{d} \pm t_{0.025} \cdot s_d$ mg./$dm.^2$ hod.	41·29 ± 2·17	39·86 ± 1·78	40·57 ± 1·36
	V	12·72	7·87	10·29
	n	12	12	24
Average	$\bar{d} \pm t_{0.025} \cdot s_d$ mg./$dm.^2$ hod.	39·61 ± 1·65	39·03 ± 1·39	39·32 ± 1·05
	V	15·30	10·80	13·05
	n	24	24	48

weight are fairly proportional to the exposure time as settled in the previous paper. On the other hand the two successive experiments 23-1 and 23-2 are distinguish by a small, yet statistically significant difference both in separate runs, and in the mean value of photosynthesis rate per hour computed from both the 3-hour and 6-hour runs. One possible reason for this discrepancy could lie in the fact that the samples employed in these experiments were not large enough to yield a true average values for the population (within the ± 5 per. cent. limit postulated above). On the other hand, it is possible that, either a change in the condition of the plants or some minor variation in the performance of the experiment, may account for the difference in photosynthetic capacity. In order to distinguish which of the two possible factors comes into operation in this case the data from Table 2 were grouped in such a manner as to eliminate differences in every group which might originate in an alternation of the conditions between the two experiments. This was achieved by treating the figures in the vertical columns of Table 2 as separate sets of data. In other words three figures from each run in each of the two experiments were combined to form a group of 12 figures. In arranging the data in such a way, figures found in sequence in the vertical columns originate from samples which differ by as many factors as possible. It can be seen that with such treatment, even results based on twelve samples are homogenous, i.e. we do not find statistically significant differences between average values computed from groups of 12 individual measurements. The difference itself will be reduced to less than ± 5% only if the average values are computed from 24 samples.

On the basis of these calculations we may conclude that from 24 plants a representative sample may be obtained for measurements accurate within ± 5%.

The small significant difference found between experiments 23-1 and 23-2 points to some shortcoming in the procedure or in the apparatus which makes it impossible to get complete

Table 4. Average values of dry weight increments in isolated leaf disks from red fodder cabbage calculated for individual groups of primary data from experiments 23-1,2-58 which are found in columns of Table 2. Dry weight increase expressed in mg. per dm.² per hour

No. of group	$\overline{d} \pm t_{0.025} \cdot s_d$	V	n	Least significant difference (P = 0·05) in %
1	38·88 ± 2·33	13·52	12	± 5·99
2	39·63 ± 2·35	13·78	12	± 5·93
3	39·63 ± 2·05	10·20	12	± 5·17
4	40·00 ± 2·44	14·82	12	± 6·10
1—2	39·25 ± 1·56	13·67	24	± 3·97
3—4	39·81 ± 1·49	12·51	24	± 3·79

agreement in the results of two successive experiments. This is quite possible since all the results quoted in this paper were obtained during a period when the separate parts of the apparatus and stages in the procedure were being continually modified in an effort to improve the method.

The procedure by which the average values of Table 4 were calculated from the data of Table 2 has been described in some detail since it suggests a way of making reliable comparative measurements of several populations in spite of some minor variations between successive runs. If, for example, the photosynthetic capacity of four varieties of one species have to be compared by this method, it is best to take samples from six plants of each variety into each of the four

Table 5. Average values of the rate of dry weight increase in samples from leaves of different crop plant varieties. The values of dry weight increase expressed in mg. per dm². per hour

No. of experiment	Reference No. of the variety	$\overline{d} \pm t_{0.025} \cdot s_d$ mg./dm². hod.	V	n	Significant difference in %
3-2-58 to 3-5-58	1	28·18 ± 1·17	9·71	30	± 4·3
	2	29·34 ± 0·98	6·85	30	± 3·4
	3	30·50 ± 0·81	4·61	30	± 2·7
	4	31·12 ± 0·96	6·49	30	± 3·1
5-1-58 to 5-4-58	3	33·76 ± 1·11	5·49	20	± 3·3
	4	32·19 ± 1·42	9·29	20	± 4·5
	5	8·60 ± 0·88	3·64	20	± 10·2
	6	9·87 ± 0·75	2·56	20	± 7·6
6-1-58 to 6-4-58	7	23·71 ± 1·35	13·14	30	± 5·7
	8	25·05 ± 1·13	9·25	30	± 4·5
	9	24·88 ± 1·52	16·85	30	± 6·1
	10	27·66 ± 1·47	16·16	30	± 5·3
12-1-58 to 12-4-58	3*	32·78 ± 1·23	8·62	24	± 3·8
	3	30·91 ± 2·15	25·97	24	± 7·0
	7	19·13 ± 1·43	11·43	24	± 7·5
	7	29·82 ± 1·27	9·07	24	± 4·3

runs and for the computation of average values to combine the data of the four runs. In this way the influence of random variations between successive runs will be reduced to a minimum. Such treatment may be extended to an even higher number of varieties, with, of course, a corresponding increase in the number of runs.

Several comparative measurements of photosynthetic capacity were made along these lines with plant populations belonging to different varieties or species of crop plants, which were grown on experimental plots under conditions described above.

The results of four such experiments are presented in Table 5. In accordance with what has been mentioned above, only results within one experimental series can be directly compared. In spite of this reservation, it may be concluded that no outstanding differences are found between the photosynthesis of individual varieties or even species. One possible exception is here represented by maize with which remarkably low values were found. This would be in accordance with claims, often found in the literature, that monocotyledonous plants are distinguished by the lower efficiency of their photosynthetic apparatus as compared with dicotyledons. It must, however, be borne in mind that both sorts of corn were rather over their culminative point in evolution at the date of the experiment; thus, it is not impossible that the very low value for photosynthetic capacity in their stands was at least partially due to physiological conditions.

The values for photosynthetic capacity in whole populations arrived at in this way have a statistical character and it is without doubt that within one experimental series and for the given conditions and plants, the comparison is valid. Minute differences may even be more significant than larger ones according to the actual variance within the populations compared. On the other hand, the primary data in hitherto quoted experiments, i.e. the dry weight increments estimated for samples representing an individual plant each, are submitted to error which makes their direct comparison only approximate. Even so, it was apparent, from mere comparison of these figures, that differences in photosynthetic capacity between individual plants are not so marked as often supposed.

In order to procure more detailed and statistically significant material for the analysis of this question, in some experiments we modified the sampling pattern so as to obtain several samples from one plant and even from a single leaf. This was, of course, possible only with sufficiently large leaves (e.g. with red fodder cabbage, sunflower) if the number of individual disks in a separately weighed sample—determined by the weighing technique—was to be preserved. The results of experimental series 7—58 are presented in Table 6 as an example of this type of experiment. For these experiments four plants of tall sunflower were selected, growing solitary on the border of the field. At the time of the experiment the plants were well advanced in blossoming so that their leaves were mature to slightly senescent, with small areas attacked by various infections or mechanically damaged. Notwithstanding, the results of the measurements are quite regular and the greatest difference found between values of photosynthetic capacity between two plants does not surpass 40%. The variability of photosynthetic capacity in individual leaves on one plant is of the same order and the largest difference in photosynthetic capacity found between two of the sixteen leaves examined (not belonging to the same plant) amounts to 80% as referred to the lower value. In the figures of Table 6 a general trend in values of photosynthetic capacity in individual leaves may be observed, related to their position on the stem; its character remains uncertain with the few cases examined, since the position of the maximal value changes from the first to the third leaf sampled.

In conclusion, some experimental results must be mentioned which demonstrate that some very important methodical problems were not considered adequately in the first phase of developing the method.

Table 6. Average values of dry weight increments in samples representing individual leaves of four sunflower plants. Primary data obtained in a 3-hour exposure expressed in mg. d. wt. dm.^{2} per hour

No. of plant	No. of leaf	1	2	3	4	Average
1	$\bar{d} \pm t \cdot s_d$ mg./dm.^{2} hod.	25·79 ± 1·04	24·29 ± 1·59	24·49 ± 2·25	21·74 ± 1·31	24·08 ± 0·65
	V	0·99	2·29	4·58	1·57	2·36
	n	16	5	6	6	24
2	$\bar{d} \pm t \cdot s_d$ mg./dm.^{2} hod.	21·14 ± 2·33	24·97 ± 2·57	27·43 ± 0·52	25·16 ± 3·92	24·67 ± 1·06
	V	4·92	6·02	0·25	13·92	6·28
	n	6	6	6	6	24
3	$\bar{d} \pm t \cdot s_d$ mg./dm.^{2} hod.	24·22 ± 2·45	26·00 ± 0·95	19·68 ± 1·38	19·94 ± 1·57	22·46 ± 0·68
	V	5·44	0·82	1·73	2·24	2·56
	n	6	6	6	6	24
4	$\bar{d} \pm t \cdot s_d$ mg./dm.^{2} hod.	25·40 ± 2·07	35·85 ± 2·15	31·12 ± 1·74	29·04 ± 1·95	30·35 ± 0·80
	V	3·91	4·21	2·76	3·45	3·58
	n	6	6	6	6	24
Average	$\bar{d} \pm t \cdot s_d$ mg./dm.^{2} hod.	24·14 ± 0·82	27·77 ± 0·77	25·68 ± 0·68	23·97 ± 0·97	
	V	3·81	3·33	2·58	5·29	
	n	24	24	24	24	

It was our intention to measure photosynthetic capacity under conditions allowing for maximal photosynthetic output in isolated leaf disks. When the apparatus was constructed it was found difficult to reach homogeneous irradiation of an intensity equal to full sunlight, over the relatively large area occupied by the disks in the chamber, and with current facilities. We decided, therefore, to work with an intensity of illumination which could be regarded as saturating for the leaves of most crop plants, i.e. 1,8 . 10^5 erg/cm². sec. It was only during actual work that some irregularities in the results led us to inquire if this condition had actually been fulfilled

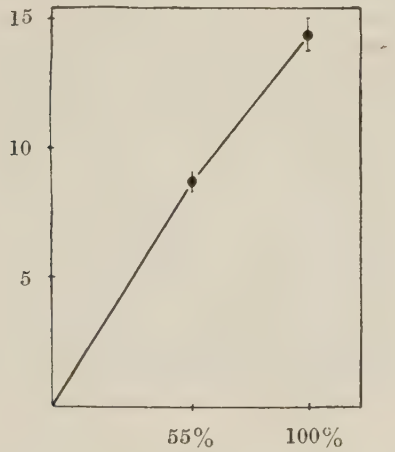

Fig. 1.

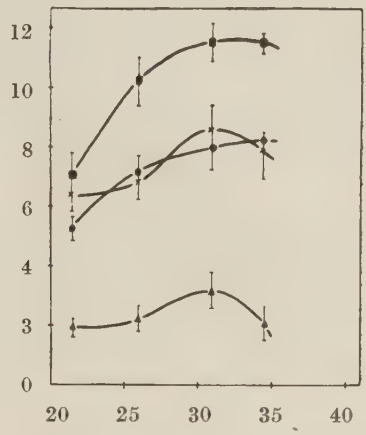

Fig. 2.

Fig. 1. Influence of light intensity on rate of photosynthesis in disks sampled from fodder cabbage leaves. Graphical presentation of results in experimental series 24-58. Abscissas: irradiation intensity in per cent of the maximal value attainable in apparatus. Ordinates: dry weight increase in disks expressed as mg. d. wt. per 9 cm². in 4 hours. Vertical lines illustrate interval covered by standard error of values represented by points ($t_{0,025} \cdot s_x$).

Fig. 2. Influence of temperature on rate of dry weight increase in isolated leaf disks in a 3-hour exposure. Abscissas: temperature in °C; ordinates: dry weight increase of disks in mg. per 9 cm². in 3 hours.

■ — red fodder cabbage × — tall sunflower
● — cucumber ▲ — early corn

In Fig. 1 the results of two such experiments performed with leaf disks from red fodder cabbage are presented. Light intensity has been reduced in one half of the chamber to 55% by means of a filter prepared photographically from a sheet of X-ray film. Parallel samples taken from the same plants and leaves were then placed in each half of the chamber and illuminated for 3 and 6 hours in each experiment. Graphical presentation of statistically treated primary data shows clearly that in the case given the intensity of illumination used in all experiments quoted in this paper was far from the saturating intensity for isolated leaf disks of red fodder cabbage. If this holds equally for samples from leaves of other plants, all measurements were performed under conditions where the light-dependent phase of the photosynthetic process becomes limiting. This fact obviously deserves further detailed examination not only in view of its consequences for the present method but also as a very important point in the study of the photosynthetic apparatus in leaves of higher plants.

Since photosynthesis of isolated leaf disks was limited primarily by the light in our experiments it is not surprising that it was relatively little dependent on temperature. In Fig. 2 results of the experimental series 11-58 are presented graphically. It is evident that the curves in this figure have the characteristic transient character specific for simultaneous limitation by tem-

perature and by light intensity with obvious predominance of the latter. It is interesting that the only exception is formed by maize, where a predominance of temperature influence is apparent. It suggests that the low rate of photosynthesis in samples from our maize plants as compared with all other varieties, was the rate of nearly light saturated photosynthesis.

Another important factor which was apparently not given due attention at the beginning of the work is the possible influence exercised by the storage of leaves detached from the plants in a dark room. This step was inserted into the procedure in order to allow the leaves to reach good turgescence, since with flaccid laminas the basic variability in parallel samples, used for

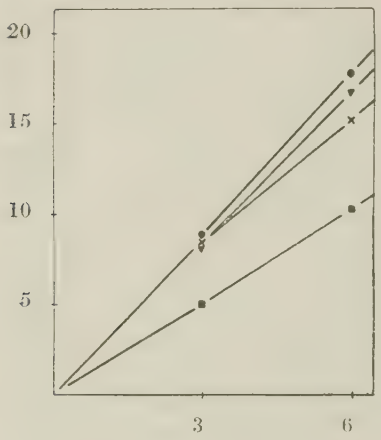

Fig. 3. Influence of duration of storing leaves in darkness on rate of photosynthesis in subsequent exposure. Graphical presentation of results in experimental series 12-58. Abscissas: Time of exposure in hours; ordinates: average dry weight increment of disks in mg. per 9 cm^2. (this is total area of 18 disks with a diameter of 8 mm.).

● — disks sampled from fodder cabbage leaves stored in darkness for 48 hours
▲ — disks from same material, time of storage 3 hours
■ — disks sampled from sunflower leaves stored in darkness for 48 hours
× — disks from same material, storage time 3 hours

the determination of dry weight increments, increases. In one of the first experiments performed by the method, different storage times were tested with leaves of red fodder cabbage and the results were found to be satisfactorily consistent. We did not realise that this may not hold for other leaves, until a systematic trend in one experimental series performed with sunflower leaves was observed, which was in apparent correlation with the length of the storage time. Results of the experiments 12-1-58 to 12-4-58, presented graphically on Fig. 3, show clearly how differently the leaves of sunflower and of cabbage respond to long storage time. Especially interesting is the fact that the rate of photosynthesis in samples from sunflower leaves remains low after 48 hours of darkness even for an exposure of six hours; this seems to exclude any induction phase and points to a permanent inactivation of some part of the photosynthetic apparatus. This question must evidently, be further examined and the storage time shortened to intervals which do not interfere with the reliability of the subsequent measurement.

Discussion

The primary source of the material from which crop yield is formed is photosynthesis of the stands. The final effect is determined both by the size of the photosynthetic apparatus (total leaf area in the stand) and by its average output per unit area (photosynthetic capacity of the leaves). In contrast with the well established positive correlation between the total leaf area of the stand and the yield, the interrelation between the yield and the average rate of photosynthesis in the leaves of different varieties is far from being satisfactorily understood.

This uncertainty is based chiefly on the lack of appropriate methods which would offer a straightforward answer to the basic question, i.e. if the average rate of photosynthesis shows

a varietal specificity and if it bears the nature of a hereditary character. In the development of such methods the most important factor will be a clearcut formulation of the problem under question. It is often not realized that we are faced here primarily with the task of finding a significant value for a quantitative hereditary character. As stated above we must decide if a reproducible value can be arrived at which would be characteristic for the photosynthetic rate in the given individual plant or variety in comparison with other plants or varieties grown under identical conditions. It is a common practice in analogous cases to grow the populations of the varieties examined under conditions as identical as possible and to compute the average value of the quantitative character from data measured on a sufficiently large number of individuals. The same should be applied to the question of average photosynthesis rate.

As a matter of fact, the rate of photosynthesis in different varieties has most often been compared under field conditions, since the establishing of a correlation between the specific rate of photosynthesis and the yield was the leading idea of all this work. With continually changing external conditions, as they are encountered in the field, only simultaneously established values can be directly compared. In this situation systematic comparative measurements, which would offer sufficiently abundant material for statistical treatment, are difficult to obtain, especially with gasometric methods, which have mostly been used hitherto. With these methods only a limited number of simultaneous measurements can be taken even if the most up-to-date techniques of gas analysis are applied.

To overcome this limitation it is possible to take systematic measurements with the limited number of objects for a long time and then to evaluate the results in correlation with simultaneously registered values of external factors (Hoover, Johnston and Brackett 1933; Verduin and Loomis 1944; Chapman and Loomis 1953). An alternative possibility is to measure the gas exchange in whole populations of the respective varieties on small plots using miniature glass-houses in place of assimilation chambers (Thomas and Hill 1937, 1949). The first method is extremely laborious and time consuming, the second one needs complex and costly installations. Thus, neither of them is adequate for comparative measurements on a large scale or for routine control.

It follows from what has been said above that a misunderstanding of the proper nature of the basic problem under consideration is characteristic of much of the work just quoted. In the search for hereditary differences in photosynthesis rate all external factors should be as constant as possible. Not only the growth of the experimental plants, but also, and in the first place, the measurement proper of the photosynthetic rate should take place under strictly comparable conditions. Variability in any set of data measured on biological material may be reduced if the work is performed under controlled conditions. In order to make use of this advantage in measuring the photosynthesis of a sufficiently large group of plants, a complex installation of the character of a phytothrone would be necessary. This again is a condition incompatible with routine work in a laboratory with standard equipment.

So the possibility which is investigated in this work remains of measuring photosynthesis of leaf samples under standard conditions. It is, of course, necessary to solve the problem of sampling so as to get a truly representative sample. In this case the dimensions of the chamber which requires air conditioning and illumination of even intensity become reasonably small.

When the relative simplicity of this type of method is taken into account, it may seem surprising that the principle has not been used more widely hitherto. The reasons for this, however, are manifold and many of the objections which can be raised have been the basis of the methodical work, referred to in this and the previous paper. Without regard to the purely technical problems, it is often argued that values measured under artificial standard conditions cannot be informative as to real values of the photosynthesis rate exhibited by the same plants under field conditions. It is by no means our

intention to deny this evident fact. But the possibility of arriving at a characteristic value for photosynthetic capacity in different varieties has, in our opinion, its own advantage for plant breeding and genetical work. It is a separate question of how a method will be found for tranformation of the results measured under standard conditions into the variable reactions of plants in the field.

It would obviously be premature to make general conclusions from the results of the work presented in this paper. A hitherto inadequately exploited and promising access to the problem of taxonomic (hereditary) specificity of photosynthetic capacity in higher plants has been devised and examined in some detail. It is by no way claimed, however, that this method will at once give the solution to such a complicated question as the inheritance of a polyvalent physiological character presents. Much work is still necessary, not only for the better illumination of this basically important problem, but also to pick out the best of the possible modifications, presented by measurements on isolated leaf disks. To this aim it has been demonstrated that it is possible to compare photosynthetic capacity reliably both when individual plants and whole populations of different sorts and species are considered, growing under one given set of external conditions, and that significant and reproducible differences can be established within and between varieties.

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Вес сухого вещества дисков листьев как критерий фотосинтеза

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Резюме

В предыдущем сообщении мы предложили определять фотосинтетическую мощь ассимиляционной ткани (потенциальную скорость фотосинтеза) листьев данного растения или данного травостоя по скорости фотосинтеза измеряемого на образцах из этих листьев в стандартных и по возможности оптимальных условиях.

Результаты настоящей работы показывают, что в скорости фотосинтеза образцов взятых из листьев отдельных растений в травостое данного сорта не существуют выразительные разницы. Это позволяет определить для растений данного сорта (в данных условиях выращивания) значение фотосинтетической мощи с удовлетворительной точностью ($\pm$ 5% при $P = 0{,}05$) при сравнительно небольшом числе входящих в опыт растений или образцов (в наших опытах от 12 до 32).

Фотосинтетическую мощь мы определяли в изолированных из листьев образцах («дисках») методом привеса сухого вещества. Пользуясь специфическими преимуществами этого метода для такого рода измерений, мы сравнивали фотосинтетическую мощь как у различных сортов и видов культурных растений, так и у отдельных растений того-же сорта. Было установлено, что травостои различных сортов принадлежащих одному виду и выращиванные в техже условиях, как правило, очень незначительно отличаются по средним значениям фотосинтетической мощи или даже между ними нельзя найти никакой статистически доказательной разницы. Даже и между сортами различных видов и семейств выразительные разницы встречаются очень редко.

Большая разнообразность результатов наблюдается при оценке фотосинтетической мощи отдельных растений данного сорта. Но и здесь разницы, как правило, не выходят за пределы $\pm$ 50%. Подобного рода различия являются типичными и для отдельных листьев одного растения.

Рассматривая все результаты приведенные в настоящей работе, надо иметь ввиду, что при измерениях фотосинтеза изолированных из листьев образцов не было достигнуто светового насыщения, вопреки нашему первоначальному намерению. Оказалось, что осуществленная в аппаратуре интенсивность освещения, $1{,}8 \cdot 10^5$ эрг/см^2 . сек, недостаточна для насыщения фотосинтетического аппарата образцов из листьев. Это, конечно, не согласуется с распространенными в литературе взглядами на характер световой кривой у листьев культурных растений.

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Distribution of the Humus Substance Fractions in Plants

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Souhrn

Z kapky roztoku nebo z lanolinové pasty s radioaktivními frakcemi humusových látek se v alkoholu (a etheru) rozpustný podíl šíří hlavně cévními svazky. Aktivní složka z huminové kyseliny se v listech zřetelně nešíří, kdežto aktivní složka fulvokyselin u některých rostlin do pletiva nevniká, ale u jiných rostlin se šíří i do vzdálenosti několika centimetrů.

Summary

The part of the radioactive fractions of humus substances which is soluble in alcohol (or in ether) spreads from drops of solution or from a mixture with lanoline paste mainly through the vascular bundles. The active component of humic acid evidently does not spread in the leaves, while the active component of fulvic acids fails to penetrate the tissues of some plants, but in others spreads to a distance of several centimetres.

Experiments with radioactive humic acids and fulvic acids have shown that the active substances are resorbed from the culture solution by maize roots, but that they spread very slowly. It takes several days for the active components of both humic and fulvic acids to reach the leaves from the roots and they only do so in very reduced intensity.

A further series of experiments was aimed to demonstrate whether the various radioactive fractions obtained from humified material can be resorbed by the leaves. The substances were applied to the leaf surfaces in aqueous

solution or mixed with lanoline paste. The preparation and activity of the substances used are described in a paper by PRÁT and POSPÍŠIL (1959).

The plants were either grown under normal illumination in a window, or in a ventilated culture cupboard with incandescent lighting; small flower pots or test-tubes containing culture solution were used.

Leaves were removed from the plants either after one to several days, or after three to four weeks, and lanoline paste was removed from them by means of filter paper. The leaves were then dried at 60 to 70° C and spread out on films to obtain autoradiograms. The leaves were usually placed between two Foma Roentgen X films, so that following exposure for a month or somewhat longer autoradiograms of both sides were obtained simultaneously.

Since differences in the results of over two hundred autoradiograms from the respective series were only slight, it is possible to summarise them briefly.

The fraction soluble in ether alcohol is usually termed bitumen. It is neither uniform nor chemically defined and therefore there can be no certainty as to which radioactive component spreads from it. Due to the effect of the alcohol (or ether) both the solution and the lanoline paste were toxic even after the solvents had been evaporated. The leaves treated with these preparations died off and dried up. Activity spread differently in different plants.

In the case of *Aesculus*, *Pelargonium* and *Phaseolus* the ether alcohol fraction did not spread from lanoline paste which had been applied to the leaves. The outline of the spot remained clear or was only slightly blurred. On the other hand, in the case of *Lycopersicum* activity spread over the leaf or through the petiole. In some cases even neighbouring leaves, particularly their vascular bundles, were clearly marked.

In view of the fact that fulvic acid and humic acid solutions dried on the leaf surfaces to form dark crusts, they were damped with water after two to three days. In maize plants *(Zea mais)* autoradiograms of first leaves which had been five days under the influence of one drop of humic acids showed only a slight advance of radioactive substances along the vascular bundles; using a counter, activity in the roots and second and third leaves of these plants was found to be only just within measurable limits. In other plants, too, *(Pelargonium, Aesculus)* the spots left by the solution or lanoline paste applied to the leaves remained in sharp outline on the autoradiograms, i.e. humic acid did not spread in the leaves.

The only exception was in the case of *Lycopersicum*, where activity spread slightly from humic acid, but only within the treated leaf, it did not spread to neighbouring leaves.

The autoradiograms of the upper and lower surfaces of the same leaf often differed. When the humic acid was applied to the upper surface the spot was more intense on the photograph of this side; since the thickness of the leaf sufficed for the absorption of the soft radiation from carbon, the film of the other side showed only a faint shadow of the spot. In so far then as the active component of humic acid spreads at all in the leaves it is, under the given conditions, below the limits or just at the limits of concentration which can be determined by means of autoradiograms.

In the experiments with fulvic acids, however, varying results were obtained

with different plants. With maize *(Zea)* and chestnut *(Aesculus)* radiograms gave no indication of noticeable spreading of the active component. Even when humic or fulvic acids were applied to young chestnut leaves which doubled or trebled their length during the experiment, i.e. in 10 to 20 days, the active spot expanded proportionately to the growth of the leaf, but its outlines remained fairly sharp and there was no clear indication of a spread of activity into the vascular bundles or the parenchyma. In leaves of *Phaseolus multiflorus*, activity from fulvic acids spread from the spots of application through the vascular bundles to the leaf margins and the parenchyma was also grey. In the case of *Lycopersicum* activity passed clearly into neighbouring leaves both through the vascular bundles and the parenchyma. In addition to the veins the leaf tips or margins were quite intensely marked on the radiograms. But in wheat *(Triticum sativum)* activity spread from the lanoline paste applied for 1 to 3 cm. along the first leaf, into the second and third leaves, which had developed later.

When fulvic acids in lanoline paste were applied to the cotyledons or leaves of flax *(Linum usitatissimum)* or pea *(Pisum sativum)*, the active component did not spread to any great extent in the leaves, so that activity was not clearly discernible in the stalk and hypocotyl; the young roots were, however, quite distinctly marked.

In general it can be stated that the active component of humic acid does not spread to any noticeable extent from drops of solution or from lanoline paste applied to leaves. On the other hand, the active component of fulvic acids spreads in some plants for distances of several centimetres. This corresponds to the size of their molecules, but further experiments will be necessary to establish whether in fact the complete molecules travel or whether they are partially split and some of their products are distributed.

There was also a striking difference between shoots of tomato *(Solanum lycopersicum)* and flax *(Linum usitatissimum)* the lower ends (cut surfaces) of which were immersed in solutions of fulvic or humic acids. In humic acid solutions adventitious roots were formed, which exhibited considerable activity on the radiograms; in spite of this, activity spread only very slightly to the stalks and leaves, and radiograms of these parts showed at the most faint shadows. On the other hand, the active substance from fulvic acid solutions spread through the whole plant and stalks and leaves were clearly marked on the radiograms. In this series adventitious roots were few or absent.

In animal organs certain elements show specific accumulation and this property is applied in diagnosis and treatment. In plants this localisation has received much less attention (e.g. Hoffmann, Toth). The question arises to what extent an analogous specific affinity exists in cases where activity is clearly localised. More frequently, heightened activity probably only indicates the direction of the current of water and solutions. Nevertheless, it is a significant phenomenon, because it can contribute to solving the question of the influence of transpiration on the rate of movement and localisation of ions in various organs. A more probable case of specificity may be the accumulation of some ions (e.g. phosphorus) and of humus fractions with C^{14} observed in meristems, in the vegetative tips of stalks and very markedly in the

adventitious roots; in the last named organs accumulation is observable as early as the stage when the lateral roots start to break through the cortex of the main root.

The autoradiogram method is, of course, considerably less sensitive than the G. M. counter, but it has the advantage of being simple and of showing the direction of spreading and the local accumulations in different tissues.

Experiments to determine the permeability of ordinary (non-radioactive) humic acids (K humate) have produced results in agreement with the above series of experiments.

The method used is essentially that employed in 1916 by S. C. Brooks in experiments on the permeability of tissues of the alga *Laminaria*. It is, however, simpler to use plexiglass tubes, or plates with an opening, instead of glass vessels. Tissue membranes, either onion epidermis *(Allium cepa)* or *Potamogeton lucens* leaves, were stretched between two sections. The lower vessel was filled with humic acid solution, the upper with several drops of water or of culture solution. At varying intervals the contents of the upper vessel was removed by suction and examined for fluorescence with UV and the reaction with p-phenylendiamine (Prát 1957) was recorded. So far there has been no evidence of the acids spreading through the tissue membrane to the liquid in the upper vessel.

It has not as yet been possible to demonstrate the presence of humus components either in the guttation sap of wheat (coleoptile and first leaf) or in the exudate from root stumps of *Impatiens sultani* and *Imp. olivieri* which had been grown in humus soil. Since, however, the sensitivity of the methods used is very unsatisfactory, it will be necessary to employ more sensitive reactions in order to enable the principle of this method to be better applied.

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Распределение фракций гумусных веществ в растениях

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Резюме

Из капли раствора или из ланолиновой пасты с радиоактивными фракциями гумусных веществ растворимая в алкоголе (и эфире) фракция распространяется прежде всего сосудистыми пучками. Активная фракция из гумминовой кислоты в листьях значительно не распространяется, активная фракция фульвокислот у некоторых растений в ткани не внедряется, однако, у некоторых растений распространяется даже на расстояние нескольких сантиметров.

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Ecophysiological Significance of the Height Gradient of the Equivalent Evaporation Power of the Atmosphere

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Souhrn

Pro výparnost ovzduší, měřenou jako relativní výpar z výparoměru, který do značné míry napodobuje fysikální poměry při transpiraci rostlinného listu (např. Picheův výparoměr), je navrhován termín ekvivalentní výparnost.

Byla stanovena výšková závislost ekvivalentní výparnosti ve výšce od 5 do 500 cm na „volné ploše", v porostu cukrovky, kukuřice, v listnatém lese mýtního stáří, v jeho obnovním kotlíku a na pasece. Čím vyšší byly absolutní hodnoty výparu, tím nižší byl výškový gradient, který s výškou ve všech případech pochopitelně progresivně klesá (obr. 1 a 2). Porosty snižují průměrnou ekvivalentní výparnost ve výškovém intervalu, sahajícím nad jejich výšku. Rozdíly relativních hodnot ekvivalentní výparnosti atmosférického prostředí, které obklopuje různé listy téže rostliny, je často podstatně vyšší než rozdíly mezi ekoklimaticky naprosto odlišnými stanovišti (např. mezi lesním porostem a pasekou).

Byla zjištěna statisticky průkazná kladná korelace mezi váhově stanovenou celodenní sumou transpirace semenáčků *Fagus silvatica* L. a *Acer platanoides* L., volně exponovaných v malých lysimetrech po celou vegetační sezonu, a mezi celodenní sumou ekvivalentní výparnosti. Pro počet dní n = 95 byl průměrný korelační koeficient r = + 0,58 ± 3 . 0,031 u buku a r = +0,56 ± 3 . 0,046 u javoru. Regresní koeficient, lineárně úměrný listové ploše, byl nízký a je důkazem silné biologické regulace transpirace.

Při současných měřeních transpirace (vážkově) listů *Syringa vulgaris* L. (n = 95) a ekvivalentní výparnosti v 10, 50 a 150 cm nad povrchem na „volné ploše" bylo ukázáno, že výškový gradient ekvivalentní výparnosti ovzduší v přirozených podmínkách ovlivňuje intensitu transpirace v různých výškách (obr. 3).

Zjištěná fakta je nutno bezpochyby spojovat se vznikem xerofytního či mezofytního charakteru listů, umístěných v různé výšce na téže rostlině, tedy také se zákonem Zalenského.

Summary

The term "equivalent evaporation power" is suggested to denote the evaporation power of the atmosphere measured as the relative evaporation from an evaporimeter which resembles to a great extent the physical conditions occurring during transpiration of a plant leaf (e.g. the Piche evaporimeter).

The height dependence of equivalent evaporation power at heights between 5 and 500 cm. in an "open area", in a field of sugar beet, maize, in a mature deciduous wood, in its small circular rejuvenation clearing and on a great clearing was investigated. The higher the absolute values of evaporation the lower the height gradient which is obviously observed to decrease progressively with height in all cases (Figs. 1 and 2). The stands decrease the average equivalent evaporation power in the height interval exceeding their height. Differences between the relative values of equivalent evaporation power of atmospheric environment surrounding different leaves of one plant are frequently considerably higher than those between ecoclimatically completely distinct localities (e.g. between a forest stand and a clearing).

A statistically significant positive correlation was found to exist between the gravimetrically determined all-day total of transpiration of seedlings of *Fagus silvatica* L. and *Acer platanoides* L., freely exposed in small lysimeters for the entire vegetation season and between the all-day total of equivalent evaporation power. For the number of days $n = 95$ the mean correlation coefficient r was $+ 0{\cdot}58 \pm 3 \,.\, 0{\cdot}031$ for the beech and $+ 0{\cdot}56 \pm 3 \,.\, 0{\cdot}046$ for the maple. The regression coefficient, which is linearly proportional to the leaf area, was low and provides evidence for the biological regulation of transpiration.

It was shown by simultaneous measurements of transpiration (gravimetrically) of leaves of *Syringa vulgaris* L. ($n = 95$) and of equivalent evaporation power at the height of 10, 50 and 150 cm. above the ground in an "open area" that the height gradient of equivalent evaporation power of the atmosphere under natural conditions affects the intensity of transpiration at different heights (Fig. 3).

The observed facts are undoubtedly to be associated with the formation of the xerophytic or mesophytic character of leaves located at different levels of the same plant, and thus with Zalensky's law.

Introduction

The evaporation power (Verdunstungsvermögen) of the atmosphere can be defined as "the tendency of the atmosphere to remove water vapour from surfaces containing free or physically bound water. The evaporation power obviously cannot be defined unequivocally as a physical factor and its physical dimension cannot be determined. It remains an un-

named, relative unit. In spite of this, the factor is extraordinarily important from the point of view of ecology. If a method is chosen for the relative estimation of evaporation power which would imitate, in a maximally standard way, evaporation analogous to that occurring during transpiration of a plant leaf, relative values are obtained comprising the effects of all principal factors of the atmospheric environment on the physical component of transpiration.

The early experience with Pichê's evaporimeter is supplemented by new findings which confirm that the measured evaporation imitates to a great extent this relative evaporation power in relation to plant transpiration (e.g. Wächtershäuser 1954, Lowry and Kudrjavcev 1958) although some papers contain evidence that the temperature conditions in particular are unfortunately quite different in an evaporation disc and in a transpiring leaf (Berger-Landefeldt 1958).

It would be useful to apply the term "equivalent evaporation power" (much like the equivalent [virtual] air humidity) to the relative values of evaporation obtained with the above-mentioned aim to imitate as closely as possible the physical conditions during transpiration. The equivalent evaporation power thus represents an approximation of the value expressing the complex of physical conditions of ecological and atmospheric environment during transpiration of the plant leaf. This equivalent evaporation power can then be used with sufficient justification for the definition of the remaining biological component of the transpiration process (e.g. for the estimation of values of relative transpiration, transpiration resistance etc.).

It is the aim of the present communication to demonstrate experimentally the ecoclimatic significance of equivalent evaporation power:

1. To point out the existence and size of the height gradient of evaporation power under natural ecological conditions;

2. to find an example of correlation between the total transpiration and values of relative equivalent evaporation power;

3. to demonstrate the relationship between the height gradient of equivalent evaporation power and the height gradient of transpiration as well as the formation of the height gradient of physiological and morphophysiological character in connection with Zalensky's law.

1. HEIGHT GRADIENT OF EQUIVALENT EVAPORATION POWER UNDER VARIOUS ECOLOGICAL CONDITIONS

The existence of considerable height gradients in the ground layer of the atmosphere is obviously not without effect on plants, on their water relations and on processes which are influenced by the consequences of a changed water balance. As these gradients are greatest in the ground layers of the atmosphere which are most important ecoclimatically as the direct living environment of plants, it appears to be of interest to devote some attention to the dependence of the equivalent evaporation power of the atmosphere on the height above ground under various ecological conditions, in various stands.

Experimental Lots and Methods

The height dependence of the equivalent evaporation power was examined in an open area without vegetation cover, in a stand of sugar-beet and above it (entire vegetation season of 1954), in a mixed beech-oak stand, in its rejuvenation opening and in a great clearing (several-day measurements at monthly intervals during the vegetation period of 1953 and 1954, see also SLAVÍK, SLAVÍKOVÁ, JENÍK 1957) and finally in a maize stand (August and September 1957).

A. Open area amidst vegetable gardens and play-fields in Praha-Dejvice.

B. Oak-beech forest stand *(Abieto-Fagetum quercetocarpinetosum)*, forest grounds Dřevíč, dept. 29c, region of Křivoklát, Central Bohemia. Average height 22 m., crown canopy 0·9, age *c.* 110 years.

C. Circular rejuvenation opening in the same stand, relative size 1·1 (24 m. in diameter); herb cover in the centre of the clearing; dense cover of *Poa trivialis* L. and *Festuca rubra* L. with average height of leaf 25 to 30 cm.

D. Great clearing area near the above stand, covered with a stage of clearing succession without marked dominance of *Calamagrostis arundinacea* ROTH and *C. epigeios* ROTH, average height of herb level 20—30 cm.

E. Sugar-beet stand [*Beta vulgaris* ssp. *esculenta* (SALISB.) GÜRKE var. *altissima* ROESSIG, Dobrovická A variety], rows 40 cm. apart, plants in rows 25 cm. apart, average height of overground part 45 cm. Locality as in A.

F. Maize stand *(Zea mays L. indurata* subvar. *purpurea)*, average height of male inflorescences 200 cm. at the time of measurement. Locality as in A.

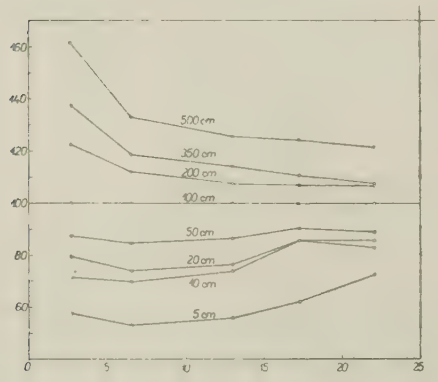

Fig. 1. Dependence of relative values of equivalent evaporation power (in per cent of simultaneous absolute value of evaporation power at 100 cm. — ordinate) at different heights above ground in an open area, on the absolute value of all-day evaporation power at 200 cm. in averages of intervals: 0—5, 5—10, 10—15, 15—20 and over 20 ml./22·7 cm². evaporation area/day — abscissa.

Results

The following results can be gathered on the basis of measurements in the open area (April to mid-November 1954 — 233 days):

The relative equivalent evaporation power at different heights (expressed as per cent relative evaporation power at 100 cm.) was shown to depend on the absolute magnitude of evaporation. The corresponding values for the individual classes of absolute evaporation at 200 cm. (macroclimate measurement) of 0—5, 5—10, 10—15, 15—20, 20—25 ml. per 22·7 cm². evaporating area per day are shown in Fig. 1. It follows from the values that the higher the absolute figures for evaporation power (measured as evaporation) the lower the height gradient at all height intervals. The size of the height gradient is determined above all by the wind which lowers the gradient, to a lesser degree also by insolation, which, when the movement of air at lower levels is small, slightly increases it. If the relative differences in evaporation power

at various heights are expressed as the actual gradient (i.e. e.g. in units with the dimension of per cent per 10 cm. height) the following values are obtained for the individual height intervals:

The all-season average (April to November 1954) with the average absolute value of evaporation being equal to 10·5 ml./day. 22·7 cm². (evaporation disc 4 cm. in diameter) at the height of 200 cm:

For the interval		Height gradient in % per 10 cm.
from	to (cm.)	
5	10	32
10	20	3·9
20	50	2·7
50	100	2·8
100	200	1·0
200	350	0·35
350	500	0·81

The period average (August and September 1957) with average evaporation being equal to 7·25 ml./day. 22·7 cm². at the height of 200 cm:

from	to (cm.)	Height gradient in % per 10 cm.
300	3500	0·06

Thus the height gradient is observed to decrease progressively with height.

Height gradients in the layer influenced by a plant stand are ecologically most important. The gradients, in their turn, directly affect the plants themselves. It is a rule that every stand decreases the relative values of equivalent evaporation power in the interval reaching from the ground up to above its average height. The size of this decrease depends on the following factors: 1. Density of the stand, i.e. the amount of limitation of convection and partly of turbulence of air inside the stand. 2. Relative humidity of the transpiration area of stand plants and their absolute transpiration. 3. The amount of restriction of incoming and outgoing radiation and thus of changes in temperature gradients between the evaporating or condensing surface and atmospheric environment.

In the sugar-beet stand the fundamental influence is limited to the lowest layers. A significant decrease in the relative value of equivalent evaporation power (increase in the height gradient) occurred in the interval from 0 to 20 cm. Evaporation power at 5 and 10 cm. is decreased markedly.

The maize stand will reduce somewhat the relative value of equivalent evaporation power even above the level of its tops (200 cm.); the decrease amounts to 20% at the height of the thickest canopy of leaves at 100 cm.,

at 50 and 20 cm. the height gradient being considerably lower. At 10 and 5 cm. the difference as compared with an open area is insignificant and the height gradient at that height resembles that of an open area; the leafless lower parts of stalk permit of more considerable air convection.

Analogously, but on a larger scale, the situation is repeated in a mature canopied deciduous forest stand. Our measurements lack data from the area of tree-tops and above them as our investigation reached only up to 500 cm. The conditions in the tree-crown region, however, can be derived from Geiger's measurements (see Geiger 1942 and 1957) of relative atmospheric humidity and of wind. It is of interest, however, that at the ground level the course of the function of equivalent evaporation power on the height coincides strikingly with conditions in the maize stand. Beginning with the height of 75 cm. in the downward direction the height gradient decreases markedly: the relative equivalent evaporation power decreases very slowly (from 64% at 50 cm. it decreases to 62% at 20 cm.) and it drops to values below 50% of simultaneous evaporation in on open area at 100 cm. in a low ground layer. Measurements in a forest stand yield separate data on day and night evaporation. At night the evaporation power is relatively high and even in the ground layer it is considerably higher than simultaneous evaporation in an open area at 100 cm. Free passage of wind through the trunk space and near the ground (herb cover is low and thin) then ensures in this region relatively high relative values of equivalent evaporation power even during the day. Therefore, in a forest stand the analogy known from the maize field in the foliage region is repeated in the tree top region on a large scale. The ground layer with sparse or completely lacking herb cover closely resembles the bare and relatively free ground layer below maize leaves.

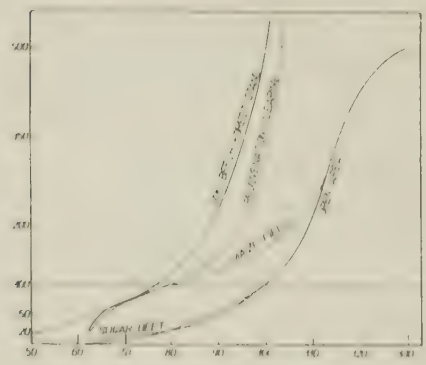

Fig. 2. Dependence of relative values of equivalent evaporation power (in per cent evaporation power at 100 cm. in an open area — abscissa) in different stands, on the height above ground — ordinate.

The height gradient of equivalent evaporation power in the centre of a small rejuvenation opening is relatively analogous to that in an open area. Nocturnal evaporation up to the height of 200 cm. is higher here than in the open area but diurnal evaporation is so much lower that the absolute values of equivalent evaporation power are about 25% lower than the parallelly proceeding height gradient in an open area. As compared with the open area, the height gradient is increased only at the level affected by grass stand in the centre of the rejuvenation opening so that there the relative equivalent evaporation power decreases to 40% and less. (On the height dependence of evaporation power in forest localities see more details in Slavík, Slavíková, Jeník 1957.)

Let us now pay more attention to examples of some height gradients and relative values of equivalent evaporation power at those heights where they can directly affect the physiological processess of plants (e.g. the origin of water deficit due to increased transpiration and its consequences):

Difference between	Height interval	Per cent difference in equivalent evaporation power	Average height gradient per cent/10 cm.
leaf-blades of a lying and standing leaf of the same sugar-beet plant	5—40 cm.	212	60
lowest and highest maize leaf	60—200 cm.	154	3·85
lowest and highest branches of a solitary shrub on the windward side	50—500 cm.	148	1·07
beech seedling and leaf at the crown base of a mature beech-tree in the stand	10—500 cm.	202	2·08

A more detailed comparison of the height gradients at ecologically important heights under various conditions shows, for example, that the leaf blade of an upright sugar beet leaf is exposed to the evaporation power of the surrounding atmosphere more than twice as much as the lower leaves of the same plant. The difference in equivalent evaporation power of the atmospheric environment of leaves of upper and lower branches of a 5 m. shrub is considerably higher (48%) than the difference between the evaporation power acting on the herb level of a deciduous forest stand and that on the clearing species (26%).

2. CORRELATION BETWEEN TRANSPIRATION UNDER NATURAL CONDITIONS AND THE EQUIVALENT EVAPORATION POWER

With the aim of verifying the correctness of correlation between total transpiration and the commonly estimated equivalent evaporation power an experiment was carried out during the entire duration of the vegetation season, using tree seedlings in small lysimeters.

Material and Methods

During the vegetation season of 1952 the transpiration of 10 seedlings of *Fagus silvatica* L. and of 5 seedlings of *Acer platanoides* L. growing in garden soil in small lysimeters (cf. Hesse 1954) was measured, the lysimeters being represented by glass beakers of 250 ml. volume. The soil surface was sealed with a layer of paraffin, vaseline and beeswax (1 : 1 : 1). Soil humidity

was adjusted daily to 60% maximum capillary capacity. The beakers with seedlings were exposed for the entire vegetation period in the experimental garden of the Institute in Praha-Dejvice and were protected from rain by a hotbed window placed horizontally about 120 cm. above the plants. The equivalent evaporation power was determined by two Piche evaporimeters [green evaporation disc 3 cm. in diameter, free evaporation area (ambilaterally) 11·7 cm².] placed at the average height of transpiring leaves of experimental seedlings.

Results

During the time from reaching full and final development of the leaf area and maturation of leaves to the beginning of first symptoms of dying (from July 1. to October 4, 1952) correlation coefficients between the all-day total of evaporation and transpiration were computed for individual plants. The correlation coefficients for the number of days of experiment $n = 95$ and average 10 experimental plants amounted to $r = + 0{\cdot}58 + 3 \,.\, 0{\cdot}031$ (max. 0·68, min. 0·40) for the beech, and $0{\cdot}56 \pm 3 \,.\, 0{\cdot}046$ for the maple.

This significant positive correlation coefficient can be compared with the correlation of evaporation and saturation deficit (WÄCHTERSHÄUSER 1945) which amounted to 0·85 for $n = 123$ during the rainless season and with the correlation between evaporation and wind velocity ($r = + 0{\cdot}45$) made by the same author.

In beech seedlings transpiration increased by 0·03 ml. per 100 cm². leaf area (lower plus upper surface) per day for each ml. water evaporated from 100 cm². evaporation area per day. This value is relatively low and at sufficient soil humidity under the given experimental conditions it indicates a high biological regulation of transpiration. (Average evaporation power during the experimental period from June to September amounted to 5·52 ml. per day and 11·7 cm². evaporation area, i.e. to 47·3 ml. 100 cm². day, the average daily transpiration being 2·01 g./100 cm². day.)

If it is realized that with all-day and even more all-season measurements of transpiration biological regulation of transpiration makes itself decisively noticeable (formation of daily water deficit and its consequences, physiological seasonal leaf ontogeny) together with experimentally hardly eliminable growth effects, our results represent a very good correlation between actual transpiration and evaporation.

3. DEPENDENCE BETWEEN THE HEIGHT GRADIENT OF EQUIVALENT EVAPORATION POWER AND TRANSPIRATION INTENSITY

It was not the aim of the described series of experiments to look for correlation values between equivalent evaporation power and the physical component of transpiration but rather to demonstrate that the height gradient of equivalent evaporation power really affects the intensity gradient of transpiration under natural conditions.

Material and Methods

At three heights above the soil ground (10, 50 and 150 cm.) the equivalent evaporation power was determined on sunny days during the morning hours (mostly between 8 and 11 a. m.) by using the Piche evaporimeter with a green disc 4 cm. in diameter and ambilateral free evaporation area 22·7 cm^2., as well as actual transpiration by means of weighing the sun-exposed leaves of *Syringa vulgaris* L., cut off immediately before determination, including the petiole, from the southern side of shrubs 15 m. from the place of measurements. The leaves were weighed on a torsion balance with 1000 mg. range and 0·5 mg. accuracy before and after a 5-minute exposure (selected according to the accuracy of weighing) in a horizontal position on a thin wire loop at the heights mentioned previously, above an unsowed bed in the middle of a large area cultivated by gardening. The results of transpiration measurements were referred to the leaf area (ambilaterally). In the days between May 22 and June 19, 1959, transpiration was determined in 95 leaves at each of the above-mentioned heights.

As it was not the purpose of the measurements to analyze the effect of evaporation power on the individual components of transpiration, the condition of the stomata and the water deficit of leaves was not investigated. It is assumed for the purposes of statistical treatment that during measurement at the given daily periods the plants possessed a relatively equilibrated water balance.

Results

Individual values of transpiration intensity were expressed in mg./cm^2. per 5 min.; the average (over a two-hour period) equivalent evaporation power was expressed in the same units. It was shown that the actual immediate transpiration of severed leaves at different values of atmospheric equivalent evaporation power at different heights depends on the intensity of this evaporation.

While the average values of equivalent evaporation power of all measurements at the above heights (10, 50 and 150 cm.) amounted to 3·37 (100%), 4·00 (119%) and 4·52 (134%) mg./cm^2./5 min., respectively, the average transpiration intensity was 1·26 (100%), 1·29 (103%) and 1·36 (108%) mg./cm^2./5 min., respectively.

In order to treat the results of measurements carried out on different days statistically, the values of transpiration for each height were expressed first in per cent of average transpiration intensity at the 10 cm. height in each measurement and these percentages were then compared statistically at three different heights. For the equivalent evaporation power, statistical treatment was accorded to the values from 12 periods (2—3 hours every time) during which measurements were undertaken, expressed as percentage of the average at 10 cm.

Fig. 3 shows the relationship between the compared values of equivalent evaporation power and transpiration at 10, 50 and 150 cm., always as $\bar{x} \pm s_{\bar{x}}$. The differences in equivalent evaporation power at the individual neighbouring heights are very significant statistically ($P = 0{\cdot}0001$). The differences between transpiration at 10 and 50 cm., and 50 and 150 cm. are not statistically significant ($P = 0{\cdot}50$), those between 10 and 150 cm. are only on the border of significance ($P = 0{\cdot}05$).

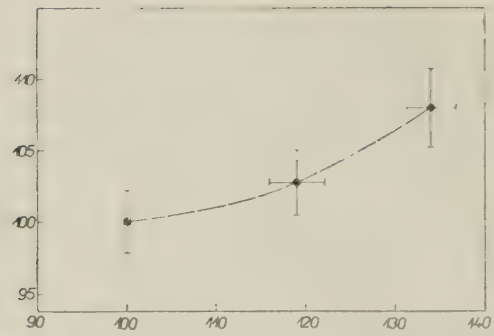

Fig. 3. Dependence of intensity of transpiration of leaves of *Syringa vulgaris* L. (in per cent of average transpiration intensity, measured 10 cm. above bare ground — ordinate), on equivalent evaporation power measured simultaneously at the same height (in per cent of average at 10 cm. — abscissa). Individual points represent values of $\bar{x} \pm s_{\bar{x}}$ at the heights of 10, 50 and 150 cm. above ground (from left to right).

The observed fact that the vertical gradient of transpiration was markedly lower than the vertical gradient of equivalent evaporation power supplies evidence for the expected biological regulation of transpiration intensity but does not represent the symptom of any indifference of transpiration in the immediate ecological conditions of the transpiring organs.

Discussion

Measurements of atmospheric evaporation power at different heights of forest and field stands and in an open area have shown that the height gradients of evaporation power, as determined by the height gradient of meteorological factors formed by the structure of a plant stand, are very great and that even within the range of height differences on individual parts of a plant they may be higher than those between ecologically quite different localities.

Simultaneous all-season measurements of transpiration and equivalent evaporation power of beech and maple seedlings have revealed that the actual transpiration under natural conditions is significantly correlated with evaporation power as long as the latter is measured in such a way as to imitate most closely the evaporation conditions of a flat green leaf.

Finally, measurements of the height gradient of intensity of immediate transpiration, the gradient of equivalent evaporation power being measured simultaneously, have shown that the height relationship of evaporation power actually affects natural transpiration.

The transpiring organs of a plant are thus at different heights of its natural height interval exposed to markedly different evaporation power of its ecological atmospheric environment. The height differences of equivalent evaporation power are surprisingly high (Fig. 2) and it is known that they actually affect the transpiration of plant organs.

These facts can doubtless be connected with the origin of the xerophytic and mesophytic character of leaves at different positions on the same plant. Morphological and physiological differentiation of leaves along the axis in the leaf spiral, in agreement with ZALENSKY's law (as stated by the named author in 1904, with reference to anatomical and anatomical-physiological properties) is not only a question of leaf ontogeny and of seasonal progress of macroatmospheric conditions, but also of the different magnitude of atmospheric evaporation power as affecting leaves of the same plant at different heights.

As concerns the water relations, two facts should be remembered. There exists a marked height dependence of osmotic pressure of the cell sap of leaves at different insertion levels in herbs*), i.e. of low plants, with leaves distributed at those heights where the height gradient is very steep. The above height dependence of osmotic pressure of cell sap has not always been established even for leaves of trees from different levels of the crown (Walter 1931, Slavík 1952). At the height usually occupied by tree crowns the height gradient is considerably less, e.g. between 250 and 500 cm. in an open area, as well as in a beech forest where it amounts to 0·4% 10 cm., whereas in the ground layer up to 40 cm. it reaches values of 60%/10 cm.

Acknowledgement.

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*) Differences in osmotic pressure of the cell sap of leaves at different levels of the leaf spiral reflect, first of all, the ontogenetic age and only then are due to different conditions under which they developed.

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Экофизиологическое значение высотного градиента эквивалентной испаряемости атмосферы

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Резюме

Для испаряемости атмосферы, измеряемой как испарение из соответствующего прибора для измерения испарения, в значительной мере имитирующего физические условия транспирации листа растения (напр. прибор Пише), предлагается нами термин эквивалентная испаряемость.

Установлена зависимость от высоты эквивалентной испаряемости на высоте от 5 до 500 см на «свободной площади», в культуре сахарной свеклы, кукурузы, в лиственном лесу продуктивного возраста, в его возобновленном котлике и на лесосоке. Чем выше абсолютные значения испарения, тем ниже высотный градиент, который, естественно, во всех случаях в зависимости от высоты прогрессивно падает (рис. № 1 и 2). Культура снижает среднюю эквивалентную испаряемость в интервале высот, превышающем их собственную среднюю высоту. Различия относительных значений эквивалентной испаряемости атмосферной среды, которая окружает разные листья одного и того же растения, зачастую значительно выше различий между экоклиматически совершенно различными экотопами (напр. между лесным насаждением и лесосокой).

Установлена статистически достоверная положительная корреляция между взвешиванием установленной суточной суммой транспирации сеянцев *Fagus silvatica* L. и *Acer platanoides* L., свободно культивированных в малых лизиметрах в течение всего вегетационного сезона, и между суточной суммой эквивалентной испаряемости. Для числа дней $n = 95$ средний корреляционный коэффициент $r = +0{,}58 \pm 3 \cdot 0{,}031$ для бука и $r = +0{,}56 \pm 3 \cdot 0{,}046$ для клена. Коэффициент регрессии, находящийся в прямой зависимости от поверхности листа, весьма низок и является доказательством сильной биологической регуляции транспирации в опыте.

В одновременных измерениях транспирации (взвешиванием) листьев *Syringa vulgaris* L. ($n = 95$) и эквивалентной испаряемости на высоте 10, 50 и 150 см от поверхности почвы на «свободной площади» указало, что высотный градиент эквивалентной испаряемости атмосферы в естественных условиях оказывает влияние на интенсивность транспирации в разных высотах (рис. № 3).

Установленные данные необходимо, несомненно, связывать с возникновением ксерофитного или мезофитного характера листьев, расположенных на разной высоте того же растения, следовательно, также с законом Заленского.

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Transmission of Wheat Striate Virus by the Eggs of the Vector *Calligypona pellucida* Fabr.

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Souhrn

Persistentní povaha viru sterilnosti a zakrslosti ovsa (VSZO) a viru proužkovitosti pšenice (VPP) byla zkoumána též v pokusu s larvami vektora *Calligypona pellucida* F., vypěstovanými z vajíček za podmínek vylučujících možnost jejich kontaminace sáním na virosních rostlinách. Vajíčka pocházela od samic z populace, u které bylo v předchozí testaci stanoveno 14,4 % individuí neinfekčních, 61,2 % nakažených VSZO, 1,9 % nakažených VPP a 22,5 % nakažených současně VSZO a VPP. Při individuálním, několikaměsíčním průzkumu infektivity u kontrolních larev, jež neměly nikdy možnost sát na virosních rostlinách, nepřenesla žádná VSZO, třebaže jím byli jejich rodiče ve vysokém stupni (celkem 83,7 %) nakaženi. Naproti tomu 14,9 % insekt přeneslo VPP, jímž byli jejich rodiče nakaženi v menším měřítku (celkem 24,4 %). Testované larvy tedy musily získat VPP od rodičovských samic transovariálně, a to ve vysokém podílu. Tato pozoruhodná vlastnost umožnila oddělit VPP od VSZO, se kterým vystupuje v ČSSR často společně. Larvy, jež absolvovaly nabývací sání na ovsu napadeném VPP, projevily proti kontrolním zvýšení infektivity o 61,6 % po uplynutí intervalu odpovídajícího 26 až 34denní cirkulační době viru v těle přenašeče a o 98,2 % po cirkulační době travající 40 až 51 dnů. V těle vektora nedochází zřejmě k interferenci mezi VSZO a VPP, neboť nakažení křísa jedním virem se nezdá být na překážku jeho nakažení virem druhým. Křížová ochrana mezi VSZO a VPP v hostitelských obilninách buď neexistuje anebo je nepatrná, poněvadž mohou trpět oběma viry současně. Příznaky jednoho viru mohou však být v různém stupni překryty symptomy viru druhého.

Summary

The persistent nature of oat sterile-dwarf virus (OSDV) and wheat striate virus (WSV) has also been examined in an experiment using larvae of the vector *Calligypona pellucida* Fabr. These larvae were grown from eggs under conditions excluding the possibility of their contamination by feeding on virus-infected plants. The eggs were from females of a population in which preliminary tests had shown that 14·4% of the individuals were non-infected, 61·2% infected with OSDV, 1·9% infected with WSV and 22·5% infected with OSDV and WSV simultaneously. Several months of individual examination of the infectivity of control larvae, which had never had the opportunity to feed on virus-infected plants, showed that none of them transmitted OSDV, although their parents were infected with it to a high degree (83·7%). On the other hand, 14·9% of the insects transmitted WSV, with which their parents were infected to a lesser degree (24·4%). Thus the larvae concerned must have acquired WSV transovarially from the female parents, and this must have taken place in a high proportion of cases. This remarkable characteristic enabled WSV to be separated from OSDV, with which it often occurs in Czechoslovakia. Larvae which went through acquisition feeding on WSV-infected oats showed, as compared with the control, a 61·6% increase of infectivity after a period corresponding to 26 to 34 days' circulation period of the virus in the vector's body and an increase of 98·2% after a circulation period lasting 40 to 51 days. Evidently mutual antagonism between OSDV and WSV in the body of the vector does not arise, since the infection of a leafhopper with one virus does not seem to prevent its infection with the other. Cross protection between OSDV and WSV in the host cereals either does not exist or is quite insignificant, because the hosts can suffer from both viruses at the same time. However, the symptoms of one virus can be masked to a varying degree by the symptoms of the other.

Introduction

The finding of oat sterile-dwarf virus (OSDV) and wheat striate virus (WSV), which caused grave damage to cereal crops in the Bohemian-Moravian Highlands from 1954 to 1956, and the main characteristics of these viruses have been dealt with already in some detail (Průša 1958a, 1958b, 1959b, 1959c, 1959d, Průša et al. 1959, Průša and Vacke 1960, Vacke 1959, 1960, Vacke and Průša 1959). The present paper presents a detailed description of that part of the investigation which led to the demonstration of transovarial transmission and the infectious properties of WSV. In addition, a short account is given of findings bearing on the question of mutual antagonism between WSV and OSDV both within the vector *Calligypona pellucida* Fabr. and in the host plants. Only brief references to this work, carried out in 1958, have so far been published (e.g. Průša 1959c, Průša et al. 1959),

Table 1. Results of test of the *C. pellucida* control larvae which transmitted WSV, although they had not fed on infected plants. (For the sake of simplicity completely negative results of infectivity tests on the remaining control larvae are omitted)

Test feeding	(17.)[1] to 29. 7.	to 1. 8.	to 13. 8.	to 22. 8.	to 1. 9.	to 5. 9.	to 17. 9.	to 26. 9.	to 8. 10.
Passage (order)	I	II	III	IV	V	VI	VII	VIII	IX
Sowing date — phase of plants at start of feeding	10. 7. 1 leaf	15. 7. 3 leaves	26. 7. 1 leaf	2. 8. 2 leaves	16. 8. 1 leaf	22. 8. 2 leaves	29. 8. 1 leaf	8. 9. 1 leaf	15. 9. 1 leaf
No. of leafhopper	Results of testing — plant diseased								
11	—	—	WSV	—	?	—[2]	—	—	WSV
14	—	—	—	—	WSV	—	WSV	—	—
15	—	—	WSV ?	WSV	—	—	WSV	—	WSV
16	—	—	WSV	—	—	—[2]	—	—	—
18	—	—	WSV	—	WSV	—[2]	—	—	—
21	—	—	—	WSV	WSV	—	WSV	—	—
28	—	—	—	—	—	—	WSV	—	—
29	—	—	—	—	WSV	—	—	—	—
44	—	—	WSV	—	—	—[2]	WSV	—	?
45	—	—	—	—	—	—	WSV	—	—
59	—	—	WSV	—	WSV	—[2]	WSV	—	—

Key: [1]) Larvae hatched from 17 to 23 July, 1958, so that test feeding started on different days.
[2]) Tested cereal was not oats, but wheat (Blanka).
— = plant stayed healthy, ? = diagnosis uncertain (plant soon died).
WSV = plant showed typical symptoms of wheat striate virus disease.

while the details have been dealt with—as in the case of the majority of the findings already published in the above-mentioned communications—only in an academic thesis (Průša 1959a).

Material and Methods

The infectiousness of both viruses found on cereals in Czechoslovakia was also tested using larvae of the leafhopper *Calligypona pellucida* Fabr. This insect is their specific vector in nature (see references above). For this purpose larvae were grown from eggs of females caught on fields round Sázava (Pelhřimov district), which is a locality regarded as one of the main primary foci of OSDV and WSV occurrence in Czechoslovakia. A preliminary individual investigation of the degree of natural infection of these leafhoppers showed that 14·4% of the individuals were non-infected, 61·2% infected with OSDV, 1·9% infected with WSV and 22·5% infected with OSDV and WSV simultaneously (see Průša 1959a, 1959b, Průša and Vacke 1960).

In growing larvae from the eggs of females from this highly infected population the following procedure was chosen in order to eliminate the possibility of contamination of the hatched larvae by feeding on virus-infected plants. Fertilised females were left from the end of June 1958 to lay eggs in the stalks of healthy barley plants, which were just at the flowering stage and therefore practically immune (age resistance). Ripe straw with the eggs (Fig. 1) was cut below the lowest nodes after three weeks and put into flower pots with healthy, newly sprouted oats plants in a silon cage. When the larvae hatched out the straw containing the eggs was quite dry and dead, so that this material could not in any case serve as a possible source of infection.

By careful daily examination of the isolation cage it was possible to collect the whitish larvae, which were very mobile and difficult to see with the naked eye, a very short time after

Table 2. Summary of results of tests with control *Calligypona pellucida* larvae (these larvae were bred under conditions excluding the possibility of feeding on virus-infected plants)

In passage	plants tested [2]	plants infected with OSDV total	plants infected with OSDV %	plants infected with WSV total	plants infected with WSV %	No. of days after hatching of larvae	No. of individuals exhibiting infection with OSDV absolute no.	with OSDV relative %	with WSV absolute no.	with WSV relative %[1]
I	74	0	0	0	0	0—12	0	0	0	0
II	63	0	0	0	0	12—15	0	0	0	0
III	55	0	0	6	10·9	15—27	0	0	6	8·1
IV	51	0	0	2	3·9	27—36	0	0	7	9·6
V	50	0	0	5	10·0	36—46	0	0	9	12·2
VI	47	0	0	0	0	46—50	0	0	9	12·2
VII	46	0	0	7	15·2	50—62	0	0	11	14·9
VIII	45	0	0	0	0	62—71	0	0	11	14·9
IX	44	0	0	2	4·5	71—83	0	0	11	14·9
Total	475	0	0	22	4·6*)					

[1]) % of 74 larvae used originally in the first passage (losses during testing are not taken into account)

[2]) The number of plants tested in each passage was determined by the number of remaining larvae

*) Total % of infected plants out of all subjected to feeding during the tests

they were hatched. Hatching started at the beginning of July. Special entomological spectacles were used for this work in order to reduce the loss of larvae through escape to the minimum. The larvae thus caught were divided into several groups which were used for concurrent experiments to demonstrate the infectiousness of both the viruses. Here mention is only made of the tests on the control group (non-infected) and of the group used in experiments on artificial infection with WSV.

There were 74 larvae in the control group. Immediately the day after they hatched out they were transferred to prepared young test plants. Following stated intervals of test feeding they were then transferred to a further cereal. The larvae from the experimental group were, on the other hand, first placed together in a silon isolation cage (Fig. 2) for acquisition feeding on several oats plants showing symptoms of WSV. They were then collected again and transferred individually to healthy test cereals—some after 6 days and some after 19 days; a few larvae, which it was not possible to catch sooner, were transferred somewhat later. In all 54 such experimentally infected insects were examined.

Testing started in the second half of July. A reliable evaluation of the results could only be made to the test feeding, which ended in the first ten days of October, because the reliability of later investigations was unfavourably influenced by autumn frosts. The following cereal species were used as test plants: oats Valečovský bílý, wheat Blanka jarní, barley Triumf, and Stupnický hanácký, rye Těšovské jarní.

Results and Discussion

Control larvae, which never were able to feed on diseased plants and were individually transferred from the middle of July to the beginning of October 1958 to new test plants nine times in all, in no case transmitted OSDV, although

Table 3a. Testing of *Calligypona pellucida* larvae from a group which acquired WSV from July 26 to August 1, 1958 on diseased oats

Passage	I	II	III	IV	V	VI	VII
Test feeding	1—12. 8.	—21. 8.	—29. 8.	—4. 9.	—15. 9.	—25. 9.	—6. 10.
Cereal, sowing date, phase at start of feeding	oats 26. 7. 1st leaf	wheat 2. 8. 2nd leaf	barley 16. 8. 1st leaf	oats 22. 8. 1st leaf	barley 29. 8. 1st leaf	oats 8. 9. 1st leaf	oats 15. 9. 1st leaf
No. of leafhopper	Results of testing — plant diseased						
1	—	—	—	—	—	—	—
2	—	—	WS	—	WS	—	—
3	—;						
4	—	—	—	OSD	—	—;	
5	—;						
6	—	WS	—	OSD	—	OSD	OSD
7	—	OSD	OSD	OSD	OSD	OSD	OSD
8	—;						
9	—	—	—	—	—	—	—
10	?;						
11	—	—	—	—	—	—	—
12	—;						
13	—	—	—	—	—;		
14	WS	WS	—	?	—	WS	—
15	WS	WS	WS	OSD	WS+OSD	OSD	OSD
16	—	—	WS;				
17	—;						
18	—;						
19	—	—	—	OSD	OSD;		
20	—	—	—	—	—	—	—

Key: — = plant remained healthy, ? = diagnosis uncertain (plant soon died), WS = plant showed symptoms of WSV, OSD = plant showed symptoms of OSDV, ; = larva missing at close of test feeding (died or escaped)

their parents were infected with this virus to a high degree (83·7%). Thus OSDV was not in this case transmitted transovarially from the parents to the offspring. On the other hand, in passages following feeding of some larvae a fluctuating percentage of the plants which they had fed on developed signs of WSV. Table 1 presents detailed results of this test. Table 2 gives a summarised account.

Larvae which showed the capacity to produce WSV disease did not, however, usually transmit this virus to each plant in the series on which they fed, but only to some of them at irregular intervals. Similar results had already been obtained with their parents (Průša 1959a, 1959b, Průša and Vacke 1960). In the whole series of passages with control larvae it was recorded that 14·9% of the individuals showed a capacity for transmitting WSV. In view of the fact that approximately 24·3% of their parents had been found to be infected with this virus, it follows that WSV must have been transmitted to a high degree by the eggs of the infected leafhoppers to their offspring.

Table 3b. Testing of *Calligypona pellucida* larvae from a group which acquired WSV from July 26 to August 14, 1958 on diseased oats

Passage	I	II	III	IV	V	VI
Test feeding	14—21. 8	—29. 8.	—4. 9.	—15. 9.	—25. 9.	—6. 10.
Cereal, sowing date, phase at start of feeding	wheat 2. 8. 2nd leaf	oats 16. 8. 1st leaf	rye 22. 8. 1st leaf	wheat 29. 8. 1st leaf	oats 8. 9. 1st leaf	oats 15. 9. 1st leaf
No. of leafhopper	Results of testing — plant diseased					
21	—	—[1]	—;[2]			
22	—	WS[1]	OSD[2]	—	OSD	—;
23	—	—[1]	—[2]	—	—	—;
24	—	—	—	—	—	—
25	—	WS	—	WS	—	—
26	—	—	—	—	—	—
27	—	—	—	—	—	OSD
28	—	—	—	—;		
29	—	—	—	—	?	—
30	—	—;				
31	—	WS	—	—	?	—
32	—	—	—	—	—	—
33	WS	—	—	WS;		
34	—	—	—	OSD	OSD	OSD
35	—	—	—	—	—	—
36	—	—	—	WS	—	—
37	—	—	—	OSD	OSD	OSD
38	WS	OSD	OSD	OSD	WS+OSD	OSD
39	—	—	—	—	—	—
40	—	—	—	WS	—	—

[1]) Test cereal was barley [2]) Test cereal was oats
For other signs see key to Table 3a

Thus wheat striate virus is in fact the first, and so far the only phytopathogenic virus on the European continent for which this remarkable characteristic has been experimentally demonstrated. Otherwise, as far as Europe is concerned, proofs of high transovarial transmittability have been recently presented for the British isles alone in respect of the European cereal (wheat) striate mosaic virus (Slykhuis and Watson 1958, Watson and Sinha 1959). This is one of the facts pointing very definitely to the identity of the latter virus with WSV.

Since transovarial transmission never took place during the above experiment while testing control larvae, it was possible to draw the conclusion that in this case successful separation of WSV from OSDV had been achieved by this means. It was then possible to carry out exact symptomatological observations of each virus separately on the different host cereals. Results of symptomatological observation of these cereal viruses have already been published (Průša 1958b, 1959a, 1959d, Průša et al. 1959, Průša and Vacke 1960, Vacke 1960).

Table 3c. Testing of *Calligypona pellucida* larvae from a group which acquired WSV from July 26 to August 21, 1958 on diseased oats

Passage	I	II	III	IV	V
Test feeding	21—29. 8.	—4. 9.	—15. 9.	—25. 9.	—6. 10.
Cereal, sowing date, phase at start of feeding	barley 16. 8. 1st leaf	oats 22. 8. 1st leaf	barley 29. 8. 1st leaf	oats 8. 9. 1st leaf	oats 15. 9. 1st leaf
No. of leafhopper	Results of testing — plant diseased				
41	—[1])	—[2])	—[3])	?	—
42	—[1])	—[2]);			
43	—[1])	—[2])	—[3])	—	—
44	—[1])	—[2])	—[3])	—	—
45	WS;				
46	—	—	—	—	—
47	—	OSD	WS+OSD	OSD	OSD
48	—	—	—	—;	
49	—	—	—	—	—
50	—	—	—	—	—
51	WS	?	—	WS	—
52	—	—	—	—	—
53	OSD	OSD	—	—	—
54	WS+OSD	OSD;			

[1]) Test plant was oats
[2]) Test plant was rye
[3]) Test plant was wheat
For other signs see key to Table 3a

During investigation of the infective capacity of larvae which were exposed to infection by feeding on WSV-infected oats, it was found that as compared with the control a much higher proportion (+ 61·6%) of insects were infected with this virus following a period corresponding to the circulation period of the virus in the body of the vector of 26 to 34 days. The percentage of WSV-infected insects increased still further during the next 17 days, for following a circulation period in the body of the vector of 40 to 51 days it attained approximately twice the value of the control (198·2%). In this way further proof was provided of the infectiousness of the agent causing wheat striation. Detailed results of tests on larvae used in the experiment on artificial infection by WSV are given in Tables 3a, 3b, 3c. They are summarised in Table 4.

It should be added that among the larvae of this group not quite a quarter of the individuals were infected with OSDV. This fact may be most readily explained by the probability that among the plants serving as the source of infection there occurred some suffering from OSDV and that this was not recognisable from the symptomatological diagnosis. In this case, too, earlier findings (Průša 1959a, 1959b, Průša et al. 1959, Průša and Vacke 1960) that *Calligypona pellucida* transmits OSDV more regularly and effectively than WSV were confirmed. The absolute number of plants infected with

Table 4. Summary of results of artificial infection of *Calligypona pellucida* larvae with wheat striate virus

Group of larvae	Passage No.	No. of plants tested[2])	No. of plants infected with OSDV total	No. of plants infected with OSDV %	No. of plants infected with WSV total	No. of plants infected with WSV %	Following circulation period in larva (days)	Total individuals exhibiting signs of infection with OSDV abs. No.	Total individuals exhibiting signs of infection with OSDV relative percentage[1])	Total individuals exhibiting signs of infection with WSV abs. No.	Total individuals exhibiting signs of infection with WSV relative percentage[1])
1.	I	20	0	0	2	10·0	6—17	0	0	2	10·0
	II	13	1	7·7	3	23·1	17—26	1	5·0	3	15·0
	III	13	1	7·7	3	23·1	26—34	1	5·0	5	25·0
	IV	12	5	41·7	0	0	34—40	5	25·0	5	25·0
	V	12	3	25·0	2	16·7	40—51	5	25·0	5	25·0
	VI	10	3	30·0	1	10·0	51—61	5	25·0	5	25·0
	VII	9	3	33·3	0	0	61—72	5	25·0	5	25·0
	Total	89	16	18·0*)	11	12·4*)					
2.	I	20	0	0	2	10·0	19—26	0	0	2	10·0
	II	20	1	5·0	3	15·0	26—34	1	5·0	5	25·0
	III	19	2	10·5	0	0	34—40	2	10·0	5	25·0
	IV	18	3	16·7	4	22·2	40—51	4	20·0	7	35·0
	V	16	4	25·0	1	6·2	51—61	5	25·0	7	35·0
	VI	16	4	25·0	0	6·2	61—72	5	25·0	7	35·0
	Total	109	14	12·8*)	10	9·2*)					
3.	I	14	2	14·3	3	21·4	26—34	2	14·3	3	21·4
	II	13	3	23·1	0	0	34—40	3	21·4	3	21·4
	III	11	1	9·1	1	9·1	40—51	3	21·4	4	28·6
	IV	11	1	9·1	1	9·1	51—61	3	21·4	4	28·6
	V	10	1	10·0	0	0	61—72	3	21·4	4	28·6
	Total	59	8	13·6*)	5	6·25*)					
Total for all groups		257	38	14·8*)	26	10·1*)					

[1]) Percentage of original no. of larvae of each group used in the first passage; [2]) number of plants used in each passage was determined by the number of surviving larvae; *) percentage of infected plants of all fed on during testing

OSDV was greater than that of WSV-infected plants, although there were fewer larvae infected with OSDV than with WSV.

Certain conclusions regarding the mutual antagonistic relationships between OSDV and WSV can be drawn from the results of this and other experiments. They show quite clearly that mutual antagonism does not occur in the body of the vector *Calligypona pellucida*, for the infection of a leafhopper with one virus does not seem in any way to hinder its infection with the other. As far as cross protection between OSDV and WSV in the host cereals is concerned, it is considered that it is insignificant or non-existent, for it is obvious that they can be attacked by both viruses simultaneously. Nevertheless, signs of one virus can to varying degrees, or completely, be masked by those of the other. This interesting question will be investigated more fully in future work.

However, present findings on the various properties of OSDV and WSV can be taken to afford adequate arguments, in spite of certain similar features, for the existence of two different viruses and not only of two strains of one virus. Results which fully confirm the above conclusions have recently been obtained by Lindsten (1959), as follows from his preliminary report on virus diseases in Sweden. He studied the nature of the so-called Bollnäs disease of oats (Bollnäs is the place where this disease has been occurring for over 20 years, although it has not been studied in detail until recently). The above author found that the leafhopper *Calligypona pellucida* is the vector of two viruses, of which one is clearly similar to OSDV and the other to WSV. Although the problem of virus diseases of cereals has not been studied in Sweden so extensively as in Czechoslovakia, it has been noted there, too, that the two viruses differ considerably in their symptomatology and that there is either no or very little cross protection between them.

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Доказательство переноса вируса полосатости пшеницы яйцами вектора *Calligypona pellucida* Fabr.

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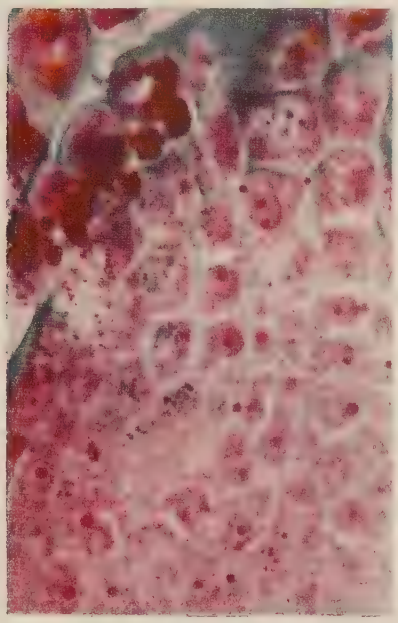
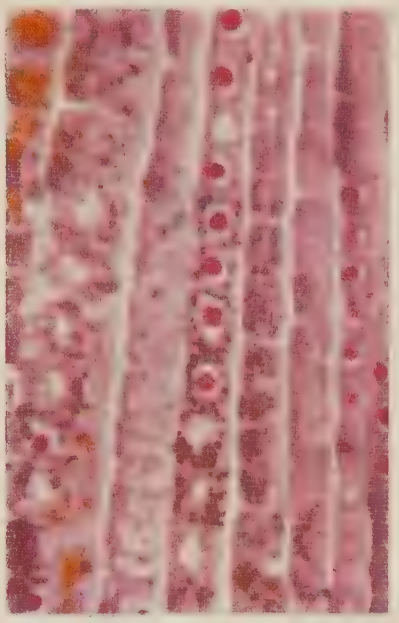
Персистентная природа вируса стерильности и карликовости овса (ВСКО) и вируса полосатости пшеницы (ВПП) изучалась также в опыте с личинками вектора *Calligypona pellucida* F. происходящими из яиц, в условиях исключающих возможность их контаминации сосанием на зараженных вирусом растениях. Яйца происходили от самок из популяции, у которой в предшествующем тесте установлено 14,4% неинфекционных особей, 61,2% зараженных ВСКО, 1,9% зараженных ВПП и 22,5% зараженных одновременно ВСКО и ВПП. При индивидуальном, несколько месяцев длившемся изучении инфекционности контрольных личинок, которые никогда не имели возможности сосать на зараженных вирусом растениях, ни одна личинка не перенесла ВСКО, несмотря на то, что им их родители в высокой степени (83,7%) заражены. Наоборот, 14,9% насекомых перенесло ВПП, которым их родители заражены в меньшей степени (24,4%). Опытные личинки, следовательно, приобрели ВПП от родительских самок трансовариально и в высокой степени. Это замечательное свойство позволило отличить ВПП от ВСКО, которые в Чехословакии выступают часто вместе. Личинки, которые прошли приобретающим сосанием на овсе, зараженном ВПП, проявляли по сравнению с контрольными повышение инфективности на 61,6% после прошествия срока, отвечающего 26—34-хдневной циркуляции вируса в теле переносчика и на 98,2% после циркуляционного срока в 40—51 дней. В теле вектора, повидимому, не имеет места интерференция между ВСКО и ВПП, так как заражение цикады одним вирусом, повидимому, не является препятствием ее заражению другим. Перекрестной защиты между ВСКО и ВПП в растениях — хозяевах или не существует или она незначительна, так как растения могут страдать от обеих вирусов одновременно. Признаки одного вируса, однако, могут в разной степени перекрываться симптомами вируса другого.



a



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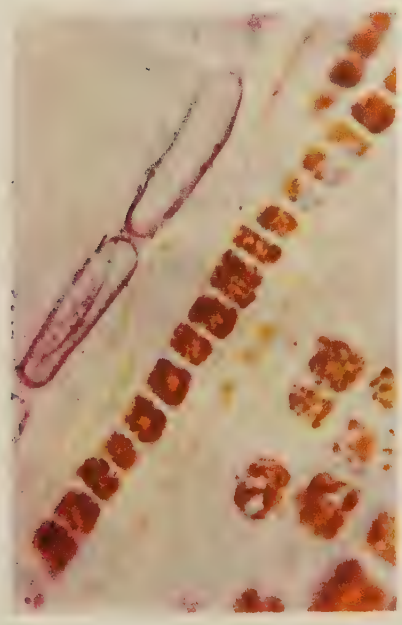


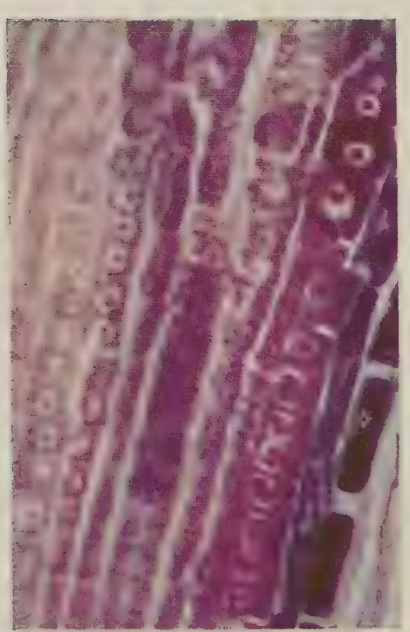
c

d

Plate 1. a. Mitochondria and starch in the root tip of *Fagopyrum*. Fixation Helly, staining Altman's acid fuchsin-tannin-methyl green. Red, mitochondria; blue-green, starch grains. Magnif. 400 : 1. b. Inclusions in the root meristem. Fixation and staining as in Fig. 1a. Magnif. 400 : 1. c. Inclusions after impregnation with silver. Method Da Fano. Magnif. 400 : 1. d. Inclusions after fixation with calcium formaldehyde, postchromed, stained with methazol blue. Blue, mitochondria. Magnif. 900 : 1.

e

f

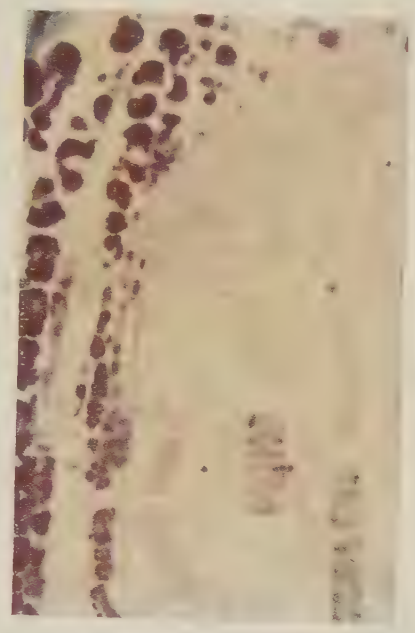


g

h

Plate 2. e. Inclusions after fixation with calcium formaldehyde without chroming, stained with methazol blue. Magnif. 400 : 1. f. The same staining as in Fig. 2e, after Baker's extraction with pyridine. Magnif. 400 : 1. g. Inclusions after freeze-drying. Coupled tetrazonium reaction. Inclusions partly destroyed, brown-orange. Magnif. 900 : 1. Violet, mitochondria. h. Inclusions stained with toluidine blue at pH 5 after extraction with trichloracetic acid. Magnif. 400 : 1.

i



j

k

l

Plate 3. i. Combined staining with PAS-Alcian blue. Magnif. 400 : 1. j. The same as c. after digestion with pectinase. Magnif. 400 : 1. k. Combined staining with PAS-Alcian blue. Section through a lateral root. Magnif. 900:1. l. The PAS reaction after blocking with phenyl hydrazine. Yellow, inclusions; violet-red, polysaccharide granules. Magnif. 400 : 1.

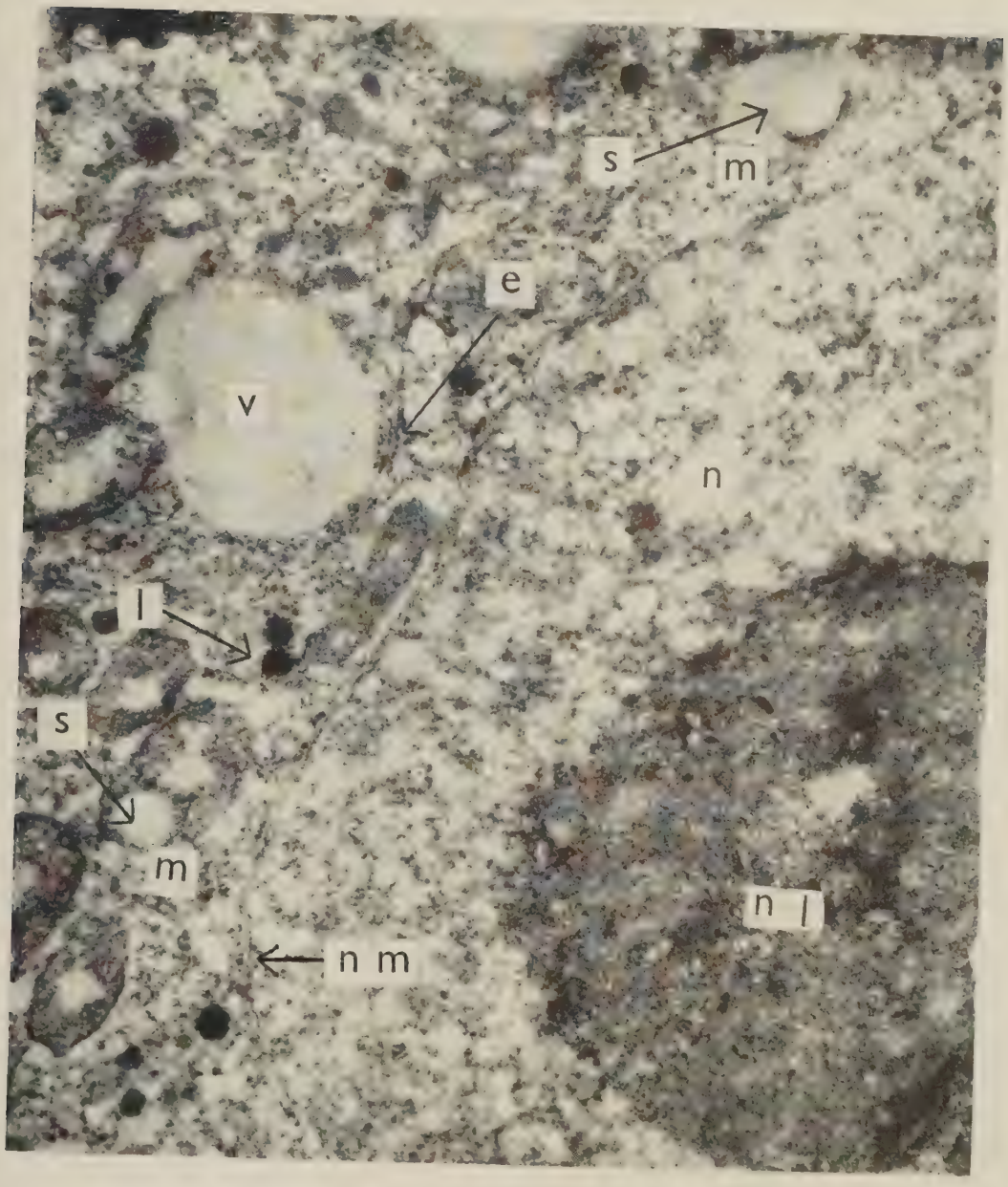


Fig. 1. An ultrathin section through a cell in the root of *Fagopyrum*. Fixation neutral formaldehyde, staining with OsO_4 (Sitte). Prim. magnif. 14 000 : 1, total magnif. 44 000 : 1.

Explanations: s = starch, ga = the Golgi apparatus, ve = vesicles, i = inclusions, v = vacuole, m = mitochondria, e = ergastoplasm, l = lipid droplets, n = nucleus, nm = nuclear membrane, nl = nucleolus.

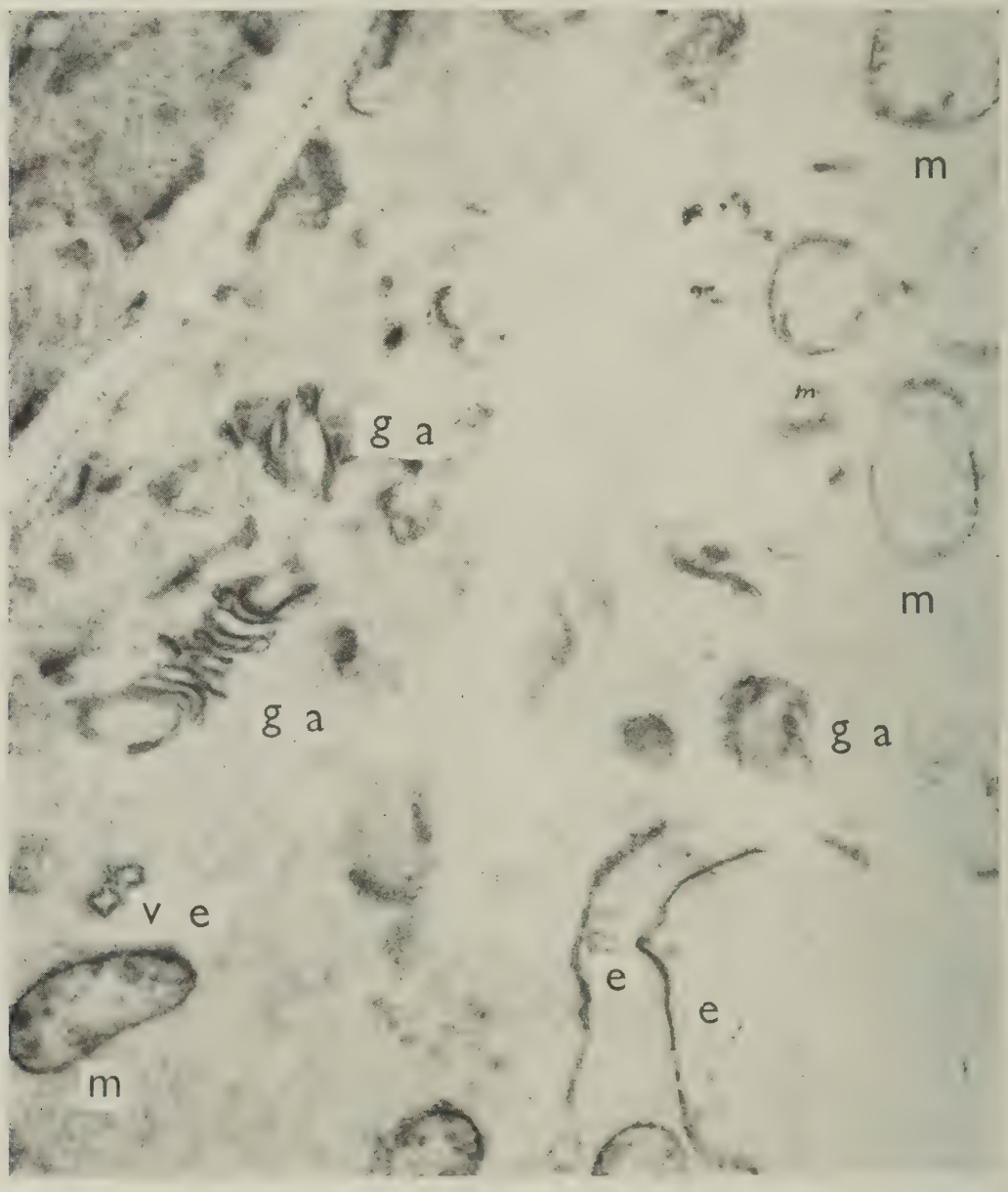


Fig. 2. Cytoplasm of a *Fagopyrum* root. Fixation in $KMnO_4$. Prim. magnif. 7000 : 1, total magnif. 20.000 : 1.

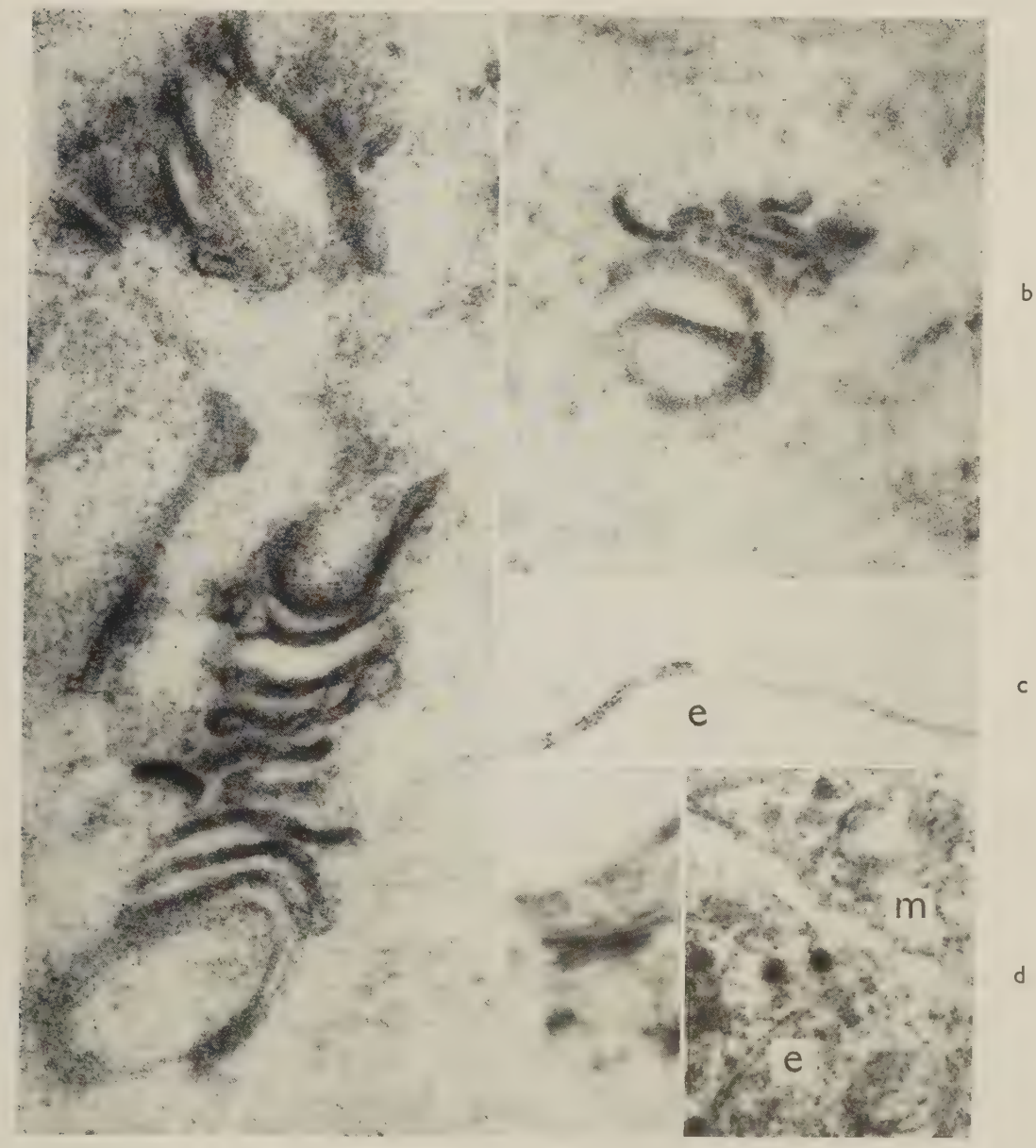


Fig. 3. a,b,e.Some forms of the Golgi apparatus in *Fagopyrum* root. c, d. Other structures. a, b c. Fixation in $KMnO_4$, d. in neutral formaldehyde, staining with OsO_4, e. OsO_4-uranyl acetate. Magnif.: a primary 20,000 : 1, total 70,000 : 1; b.c. primary 20,000 : 1, total 90,000 : 1, d. primary 14,000 : 1, total 42,000 : 1; e. primary 14,000 : 1, total 36,000 : 1.

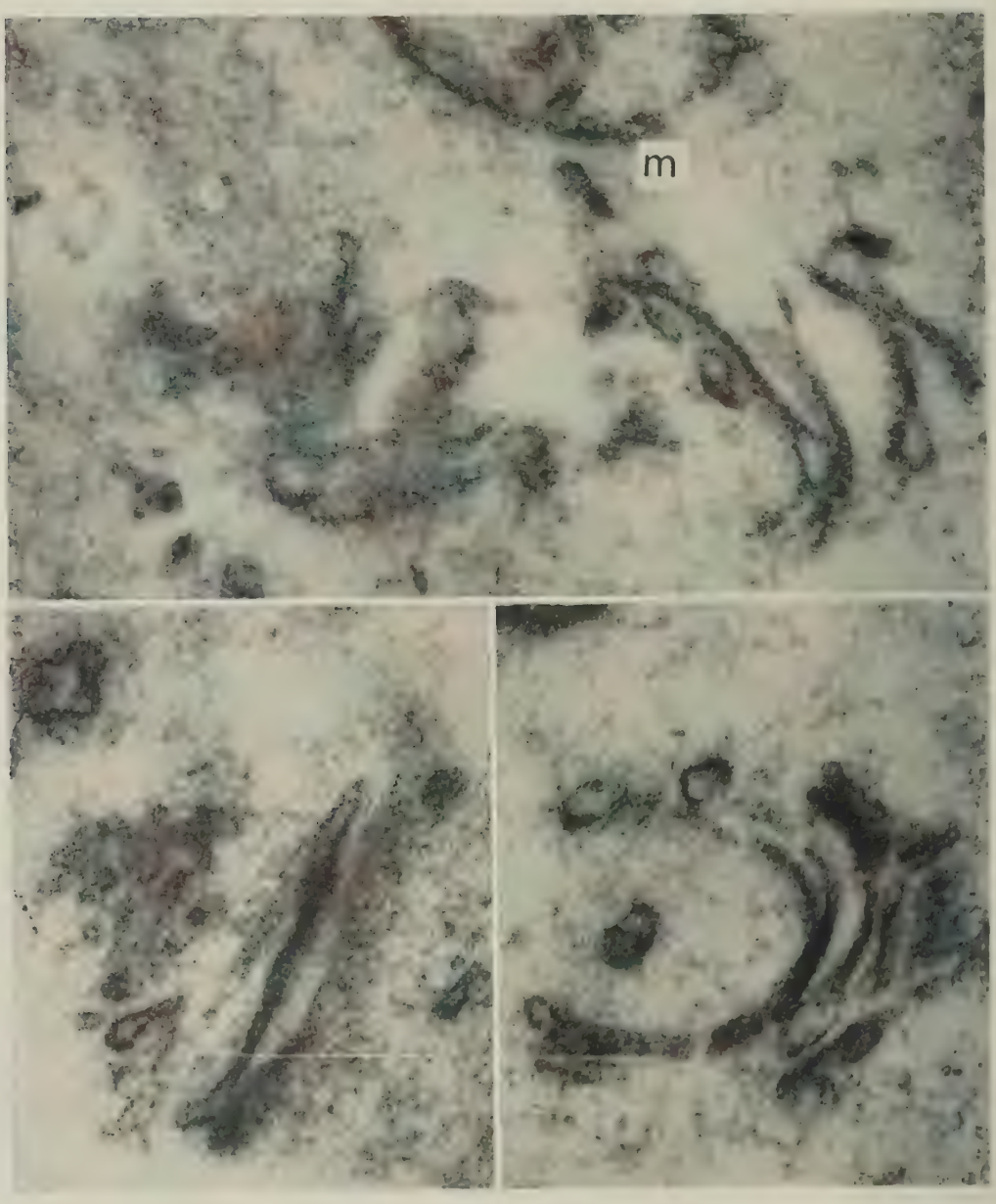


Fig. 4. Some forms of the Golgi apparatus. Fixation in $KMnO_4$. Primary magnif. 18,000 : 1 total 70,000 : 1.

Fig. 5. The ergastoplasm. Fixation in $KMnO_4$. Primary magnif. 20,000 : 1, total magnif. 70,000 : 1.

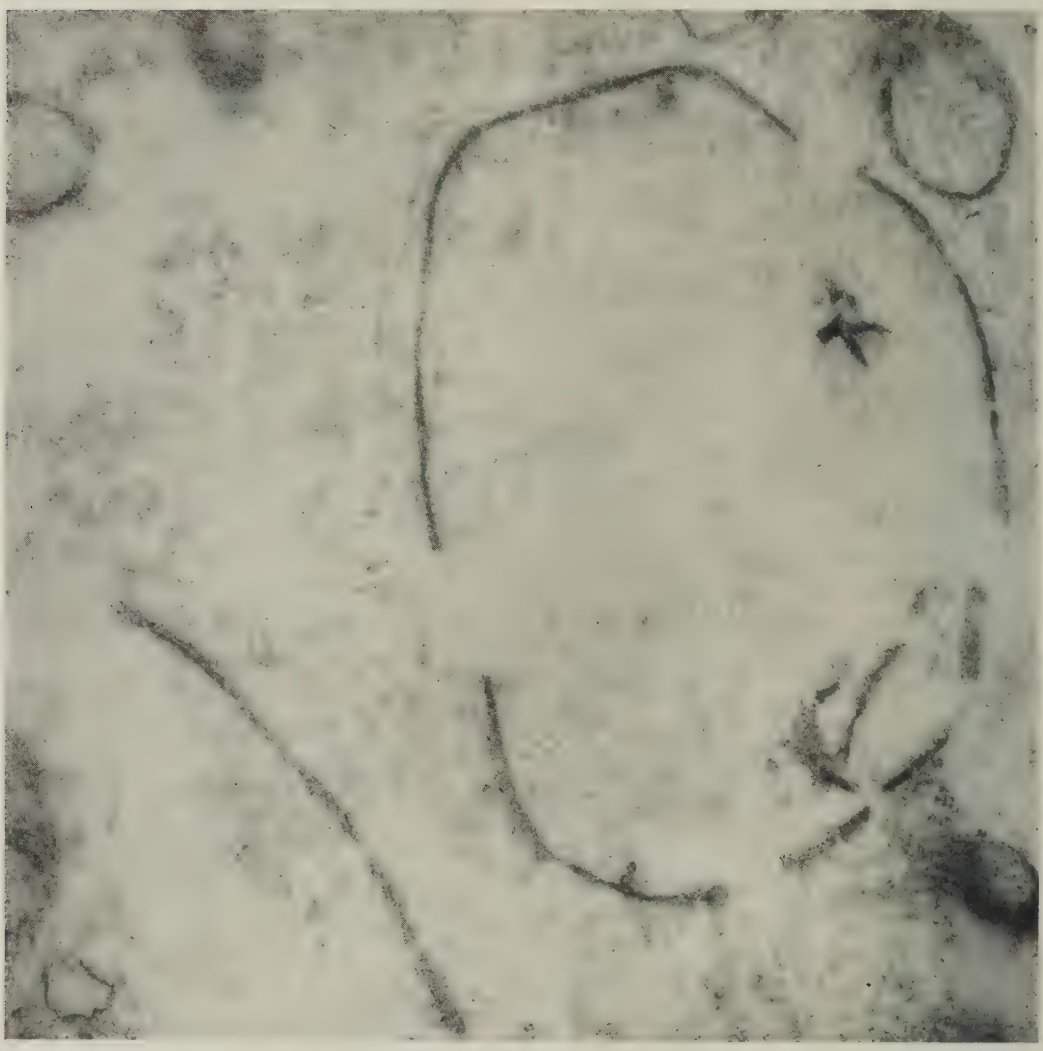


Fig. 6. The ergastoplasm. Fixation in $KMnO_4$. Primary magnif. 18,000 : 1, total magnif. 70,000 : 1.

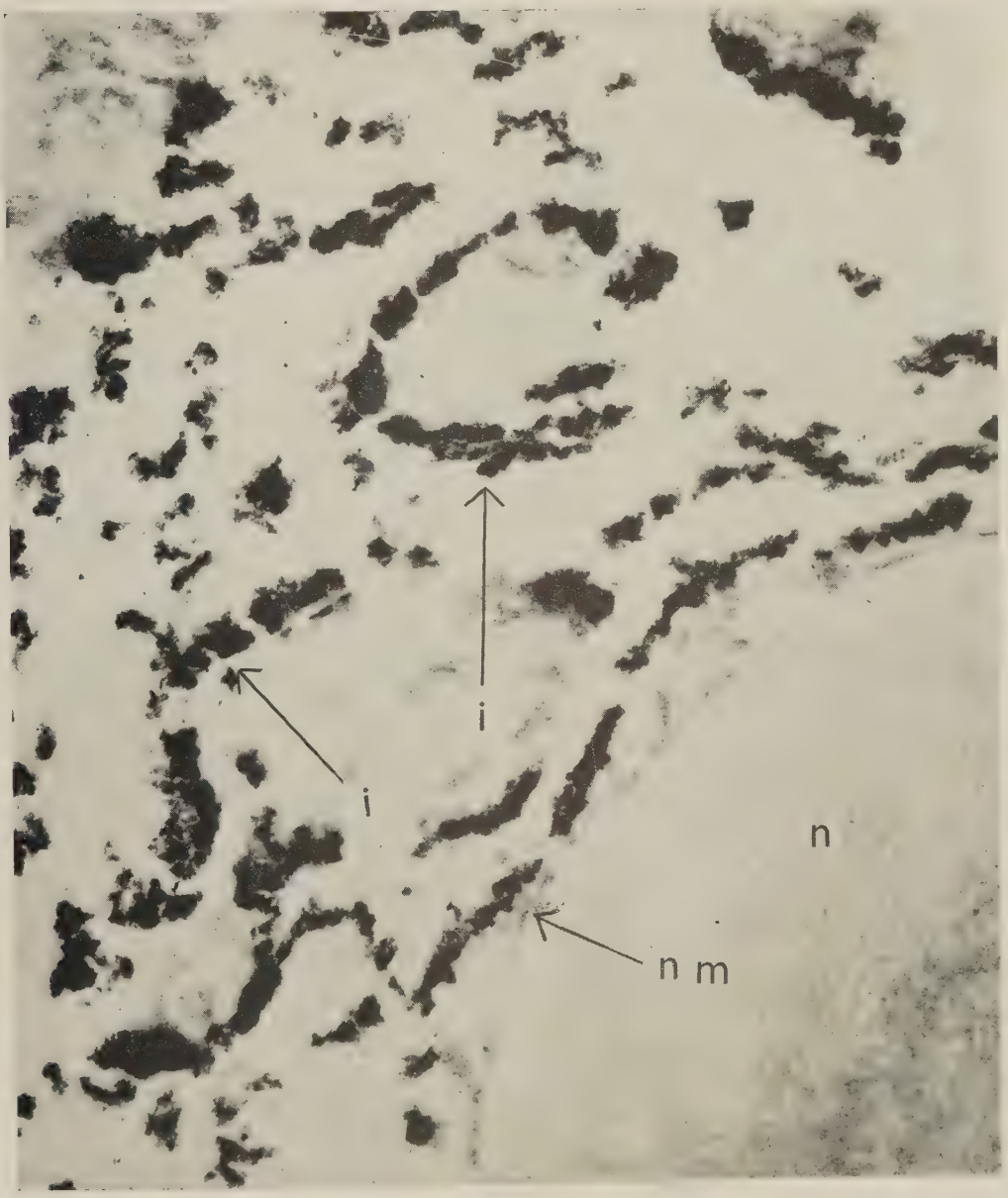


Fig. 7. Inclusions. Fixation in $KMnO_4$. Primary magnif. 7000 : 1, total magnif. 24,000 : 1.

Fig. 1. Modification of entomological exhaustor used in experiments with individual transference of vectors.

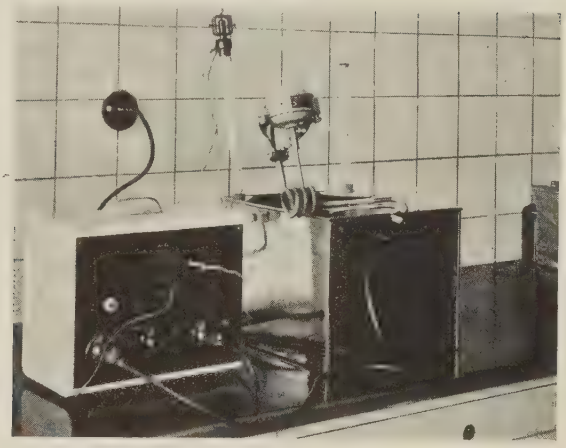


Fig. 2. Apparatus used in experiment on thermal inactivation of WSV and OSDV in leafhoppers (electronic contactor, Vertex, water bath, heating unit, electric mixer, control thermometer).

Fig. 3. Spots following feeding by aphids on young oats; left, untreated control.

Fig. 4. Wheat leaves infected by WSV; left, leaf from healthy plant.

Fig. 5. Barley leaves infected with WSV; right, healthy leaf.

Fig. 6. Striation on glumes of spikes in oats panicles.

Fig. 7. Necrosis on leaves of barley var. Triumf caused by WSV; right, healthy leaf.

Fig. 1. a, b) *Lycopersicum esculentum* leaf treated with lanoline paste containing the ether-alcohol fraction of humus substances (May 20 to June 7, 1958). The active component spreads through the vascular bundles to the neighbouring leaves.
c) *Aesculus hippocastanum* leaf treated with lanoline paste containing the fulvic acid fraction (January 28 to February 15, 1959). Outlines of the spots remain sharply defined, activity does not spread.
d) *Lycopersicum esculentum* leaf treated with lanoline paste containing the fulvic acid fraction (May 20 to June 18, 1958). Activity spreads to neighbouring leaves.

Fig. 2. a, b) Upper (a) and lower (b) surfaces of *Pelargonium zonale* leaf, the upper surface of which was treated with lanoline paste containing active humic acid (January 28 to February 5, 1958). The spot remained clearly defined, activity did not spread at all.

c) A drop of radioactive humic acid solution was applied to an *Aesculus hippocastanum* leaflet. After eight days (March 14 to March 22, 1958) the outlines are sharp, acid has not penetrated into the tissues and has not spread.

d) Lanoline paste with radioactive humic acid applied to an *Aesculus hippocastanum* leaflet (January 28 to February 15, 1959). The edges of the spot remained clear, activity did not spread.

e, f) *Aesculus hippocastanum* leaflet treated with lanoline paste containing humic acid (March 15 to April 15, 1959). The leaf grew to twice its size, the spot extended proportionately, but activity did not spread. Upper and lower surfaces of leaf.

a *b*

Fig. 3. a, b) *Aesculus hippocastanum* leaflet treated with paste containing active acids. After two months (April 14 to June 7, 1958) the leaves doubled their original length, the treated patches increased and diffused in proportion to growth, but activity did not spread to the leaf tissues either from fulvic acids (a) or from humic acids (b).

Fig. 4. a, b) Cut shoots of *Lycopersicum esculentum* were placed with their cut ends in radioactive solution (May 22 to June 1, 1959).
a) Humic acid activity spreads very slightly from very active adventitious roots and at the most is only very faintly discernible at the leaf margins.
b) Activity spreads from fulvic acids throughout the plant.

a *b*

Fig. 5. a, b) Cut flax stems *(Linum usitatissimum)* were placed with their cut surfaces in radioactive solution (June 12 to June 22, 1959).

a) Humic acid activity spreads throughout the plant, but very weakly. It accumulates distinctly at the leaf tips.

b) Fulvic acid activity spread in quite high concentration throughout the plant; here, too, it clearly accumulates at the leaf margins and tips.

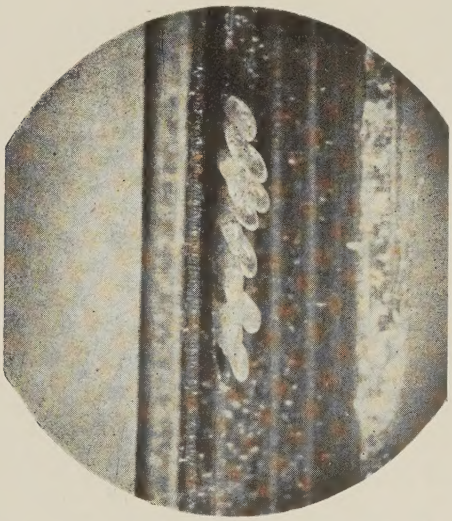


Fig. 1. Group of *Calligypona pellucida* eggs in a cut barley stalk. Orig. Průša, photo Novák.

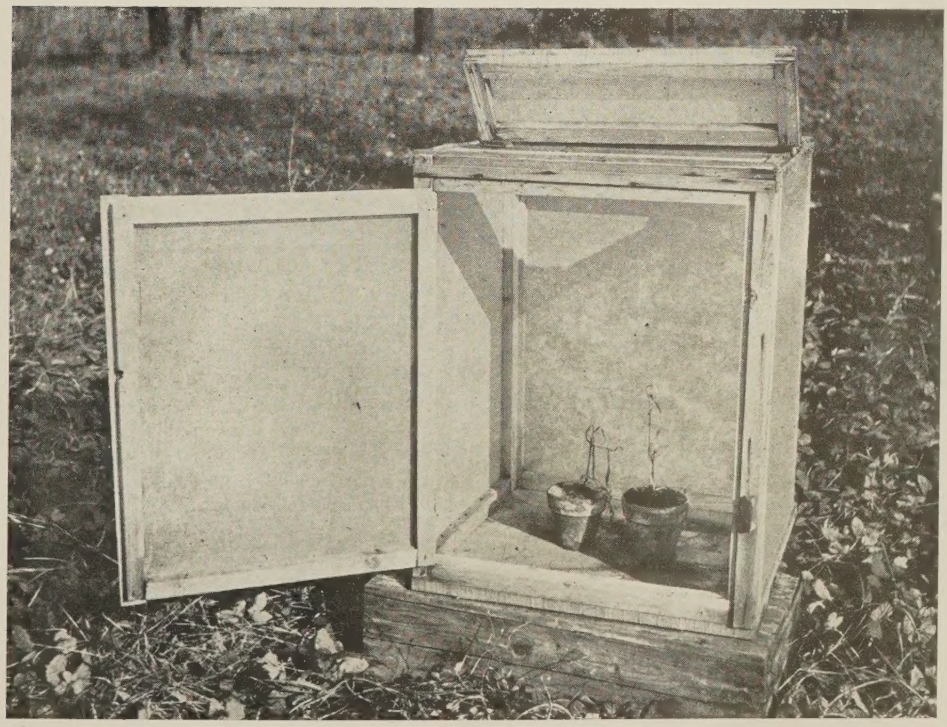


Fig. 2. Silon isolation cage used for acquisition feeding in experiments on the artificial infection of *Calligypona pellucida*. Orig. Průša, photo Novák.

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